



## The Effect of Aqueous Extract of Saffron (*Crocus sativus* L. *Stigma*) on the Behavior of *Salmonella* Typhimurium in A Food Model during Storage at Different Temperatures

Soghra Valizadeh; DVM<sup>1</sup>, Mir-Hassan Moosavy; PhD<sup>\*1</sup>, Alireza Ebrahimi; MSc<sup>1</sup>, Afshin Akhondzadeh Basti; PhD<sup>2</sup>, Razzagh Mahmoudi; PhD<sup>3</sup> & Seyed Amin Khatibi; PhD<sup>1</sup>

<sup>1</sup> Department of Food Hygiene and Aquatic, School of Veterinary Medicine, University of Tabriz, Iran.

<sup>2</sup> Department of Food Hygiene, School of Veterinary Medicine, University of Tehran, Iran.

<sup>3</sup> Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.

### ARTICLE INFO

#### ORIGINAL ARTICLE

#### Article history:

Received: 20 Feb 2021

Revised: 19 Jun 2021

Accepted: 18 May 2021

#### \*Corresponding author:

moosavy@tabrizu.ac.ir

Department of Food Hygiene and Aquatic, School of Veterinary Medicine, University of Tabriz, Iran.

Postal code: 5166616471

Tel: +98 4136378742

### ABSTRACT

**Background:** Given the concerns about the use of chemical preservatives in food, the consumers and producers have been interested in natural alternatives, such as plant essential oils and extracts. Since there are limited studies about the effect of saffron (*Crocus sativus* L.) on the behavior of foodborne pathogens in food models, this study aimed to determine the inhibitory effect of aqueous extract of saffron stigma on the growth behavior of *Salmonella* Typhimurium (*S. Typhimurium*) in commercial barley soup (as a food model) during storage at different temperatures.

**Methods:** The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extract were determined against *S. Typhimurium* using broth microdilution method. The growth of *S. Typhimurium* was investigated in the presence of this extract in commercial barley soup during 12 days of storage at 10, 20, and 30 °C. **Results:** The MIC and MBC values for saffron extract against *S. Typhimurium* were 100 and >200 mg/ml, respectively. Also, the saffron extract at a concentration of 200 mg/ml and temperature of 10 °C had the highest inhibitory effect on the growth of bacteria in commercial barley soup during storage. **Conclusion:** According to the results of this study, the antimicrobial effect of this extract increased in a dose-dependent manner against this bacterium. Therefore, the use of proper concentrations of this extract together with appropriate storage temperature can have an appropriate inhibitory effect on the growth of this bacterium, improving food safety shelf life.

**Keywords:** Saffron; *Crocus Sativus* L; Antimicrobial; *Salmonella*

### Introduction

Various microorganisms, including gram-positive and gram-negative bacteria, as well as fungi, cause a variety of infections in humans.

Over the years, effective antimicrobial substances have been developed to overcome pathogenic microorganisms. However, in recent years,

**This paper should be cited as:** Valizadeh S, Moosavy MH, Ebrahimi A, Akhondzadeh Basti A, Mahmoudi R, Khatibi SA. The Effect of Aqueous Extract of Saffron (*Crocus sativus* L. *Stigma*) on the Behavior of *Salmonella* Typhimurium in A Food Model during Storage at Different Temperatures. *Journal of Nutrition and Food Security (JNFS)*, 2022; 7 (1): 37-45.

microorganisms resistance to common antimicrobial drugs has increased, leading to an urgent need for novel antimicrobials (Kalaivani *et al.*, 2012, Khatibi *et al.*, 2020a, b). According to the reports of the centers for disease control and prevention (CDC), 76 million people in the United States are infected with foodborne pathogens annually, leading to 225,000 cases of hospitalizations and 5,000 cases of death (Oussalah *et al.*, 2007).

From ancient times, plant extracts have been used for various purposes, such as improving the flavor of foods and beverages, and the treatment of various diseases. Since public concerns have increased about the side effects of chemical preservatives in recent years, the use of plant extracts has been known as a promising way to increase the shelf life of food, due to their natural origin and more safety compared to chemical preservatives (Khatibi *et al.*, 2015, Khatibi *et al.*, 2017, Khatibi *et al.*, 2018, Moosavy *et al.*, 2017, Moosavy *et al.*, 2018, Pandey *et al.*, 2017, Santos-Sánchez *et al.*, 2017).

Saffron (*Crocus Sativus* L.), belonging to the Iridaceae family, is a perennial plant that is traditionally used to improve the taste and flavor of food (Raj *et al.*, 2015). The dried stigma of this plant is usually used in the food industry as an aromatic spice and coloring agent (Hill, 2004, Mzabri *et al.*, 2019). According to the findings of previous studies, the extract of this plant has antimicrobial, antioxidant, and anticancer properties (Amoozadeh *et al.*, 2016, Karimi *et al.*, 2010, Milajerdi *et al.*, 2016, Parray *et al.*, 2015). Saffron contains over one hundred and fifty different volatile compounds. The main constituents of this plant are Crocin, Picrocrocin, and Safranal, which are effective in color, taste, and smell of saffron, respectively. Each of these compounds plays an important role in the antioxidant and antimicrobial properties of saffron (Ökmen *et al.*, 2016).

Different types of soup have been reported as a source of salmonellosis outbreak in Germany (Geiss *et al.*, 1993), Vietnam (Vo *et al.*, 2014), and USA (Hedican *et al.*, 2009). In Europe, the

consumption rate of soup per capita is estimated at about 0.8 kg/person/year (Dionisi and Oldring, 2002). Commercial/homemade barley soup is one of the most popular types of soup throughout the world. Due to having special and different compounds, such as meat, onion, carrot, parsley, and barley, it is a rich source of high-quality protein, vitamins, and minerals. It provides an appropriate medium for the growth of food-borne pathogens and spoilage microorganisms (Pajohi *et al.*, 2010, Shahbazi *et al.*, 2017). In recent years, barley soup has been used by many researchers as a food model to study the effect of antimicrobials on the growth of pathogenic foodborne microorganisms (Ahmadi *et al.*, 2017, Moradi and Sadeghi, 2017, Pajohi *et al.*, 2010, Shahbazi *et al.*, 2017, Sharafati Chaleshtori and Fallah, 2019). Therefore, this study aimed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous extract of saffron stigma on *Salmonella* Typhimurium, as the most important foodborne pathogen. Also, the commercial barley soup was used as a food model to investigate the antimicrobial effect of the extract on the behavior of this bacterium at different temperatures of storage.

### Materials and Methods

**Bacteria:** *Salmonella enterica* subspecies *enterica* serovar Typhimurium (PTCC: 1709) was used for this study. The lyophilized bacteria were prepared from the collection center of industrial microorganisms (Iranian Research Organization for Science and Technology, Iran). The bacterium was cultured consequently in the nutrient broth (Merck, Germany) at 37 °C for 24 hours. The second culture was then mixed with sterile glycerin in a ratio of 5: 1 and stored at -20 °C to be used during the study (Pintado, 2011).

**Preparation of bacterial inoculums:** Briefly, 100 µl of the bacterial suspension was transferred to 10 mL of the nutrient broth and incubated at 37 °C for 24 h. This culture was repeated under the same condition. Using 0.1% sterile peptone water, serial dilutions of the culture were prepared, and the

bacteria were cultured on the surface of nutrient agar (Merck, Germany). After incubation of plates at 37 °C for 24 h, colonies were counted. Using the surface culture method, the number of bacteria was calculated as colony-forming unit (CFU) per mL of the culture medium.

*Extraction of aqueous extract:* The saffron was collected from a field in Gonabad city. The stigmas of the plant have been fully dried in the shade and under a gentle current of air. To prepare the aqueous extract, 13.8 g of clean and crushed saffron stigmas were mixed with 50 ml of distilled water and boiled for 20 minutes. The resulting mixture was passed three times through filters with large to small porosity degrees. The filtered solution was placed in a water bath at 50 °C until the water evaporated completely. The residual dry matter was distributed in sterile microtubes and stored in a refrigerator for the next experiments (Pitsikas and Sakellaridis, 2006).

*Determination of minimum inhibitory concentration of the extract by microdilution broth method:* To determine the MIC of the extract, 96-round well microplates with a volume of 300 µl were used. Different extract concentrations of 12.5, 25, 50, 100, 200 mg/ml were prepared using distilled water. Firstly, 200 mg of dried saffron extract was dissolved in 1 ml of distilled water. The resulting solution was passed through a 0.45 µm filter and used for the preparation of lower dilutions. Then, 20 µl of the extract with the desired concentration and 160 µl of broth medium were poured into each well. Twenty (20) µl of bacterial suspension was added to each well. The final bacterial concentration was 10<sup>5</sup> CFU/ml (the exact number of bacteria was determined by surface culture and colony counting). A well containing 180 µl of nutrient broth medium and 20 µl of bacteria was considered as the positive control. Also, a well containing the nutrient broth was used as the negative control. To control possible contamination of the extract, a well containing 20 µl of the extract and 180 µl of nutrient broth medium was also used. The contents of the microplate were mixed for 2 minutes using a microplate shaker. After incubating the microplates

at 37 °C for 24 h, the wells were visually monitored for the presence of turbidity. The minimum concentration of the extract that inhibited bacterial growth was considered as the MIC. The experiments for detecting the MIC value were performed in triplicates (Khatibi et al., 2018, Wadhvani et al., 2009).

*Determination of minimum bactericidal concentration of the extract:* For this purpose, wells in which bacterial growth was inhibited were used for this experiment. A sterile swab was impregnated with the content of each well and cultured on the surface of nutrient agar. The culture was incubated for 24-48 h at 37 °C, and the count of bacteria was counted. The minimum concentration of extract that inhibited the growth of 99.9% of bacteria was considered as the MBC. These experiments were performed in triplicates (Khatibi et al., 2017).

*Preparation of substrate:* Each package (85 g) of commercial barley soup (Nestle, Iran) was added to 1 liter of distilled water according to the manufacturer's guidelines, and were heated for 15 min. The mixture was passed through a strainer and distributed in microtubes. Finally, it was sterilized at 121 °C for 20 min (Moosavy et al., 2017).

*Addition of the extract to soup and storage at different temperatures:* After sterilization and cooling of the barley soup, 100 and 200 µl of aqueous extract was added to 900 and 800 µl of soup, respectively. Then, 100 µl of bacterial suspension was added to the mixture with a final concentration of 10<sup>5</sup> CFU/ml. A control sample was also prepared. The samples were incubated at 10, 20 and, 30 °C for 12 days. To evaluate the antibacterial effect of extract against *S. Typhimurium*, the bacterial colonies were counted after 0, 1, 2, 3, 6, 9, and 12 days. For colony count, serial dilutions of the samples were prepared using 0.1% peptone water and were cultured on the surface of nutrient agar plates. They were incubated at 37 °C, and the count of bacteria was enumerated after 24 h. This experiment was performed in triplicates (Moosavy et al., 2017).

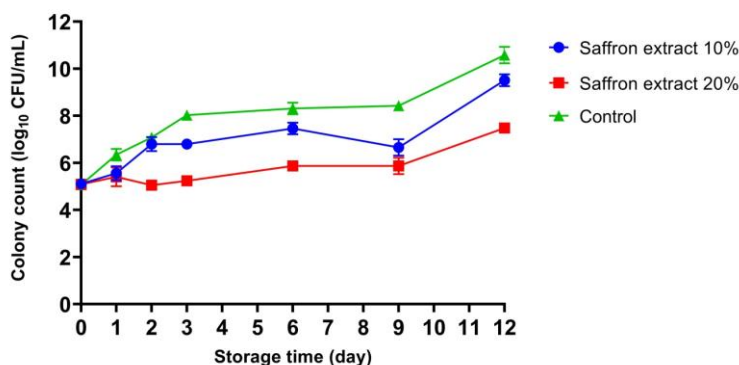
**Data analysis:** The SPSS software version 19 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis of data, and p-value less than 0.05 was considered statistically significant.

**Results**

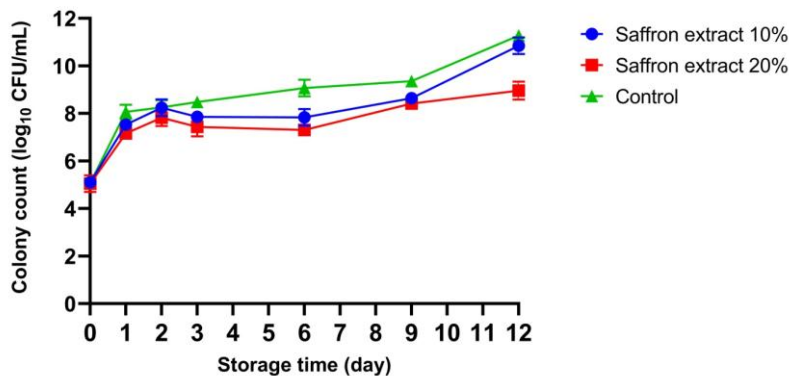
**Minimum inhibitory and bactericidal concentrations of the extract:** After adding the desired amounts of extract and bacteria to each well and incubating microplate at 37 °C, it was found that a concentration of 100 mg/ml of the extract can inhibit the growth of *S. Typhimurium*. Therefore, this concentration was considered as the MIC against this bacterium. After the culture of the contents of the clear wells, it was found that none of the studied concentrations could kill this bacterium. Therefore, the concentrations above 200 mg/ml were determined as the MBC.

*The effect of the extract on the behavior of Salmonella Typhimurium in commercial barley*

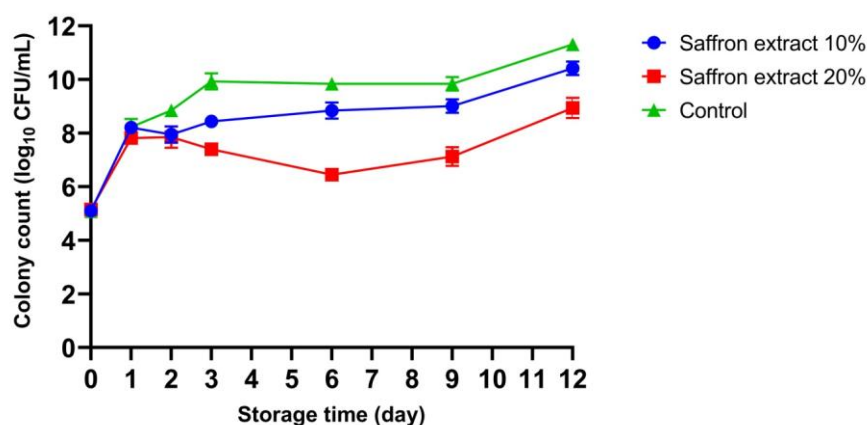
*soup at different temperatures:* To investigate the inhibitory effect of aqueous extract of saffron stigma on the growth of the bacteria in barley soup during storage, the bacterial count was enumerated at the selected days and temperatures. The results of the bacterial colony count are shown in **Figures 1-3**. Bacterial count during the storage period of barley soup was affected by the extract in a dose-dependent manner. By increasing the concentration of the extract from 100 to 200 mg/ml, the bacterial count significantly ( $P < 0.05$ ) decreased after 12 days. Storage temperature also had a significant effect ( $P < 0.05$ ) on the bacterial count. After 12 days of storage at 10 °C, the concentration of 200 mg/ml of the extract decreased the bacterial count by  $3.1 \pm 0.15$  log compared to the control sample, while this difference was  $2.32 \pm 0.23$  and  $2.37 \pm 0.18$  log at 20 °C and 30 °C, respectively.



**Figure 1.** Behavior of *Salmonella Typhimurium* in commercial barley soup using different concentrations of aqueous extract of saffron stigma during 12 days of storage at 10 °C



**Figure 2.** Behavior of *Salmonella Typhimurium* in commercial barley soup using different concentrations of aqueous extract of saffron stigma during 12 days of storage at 20 °C



**Figure 3.** Behavior of *Salmonella Typhimurium* in commercial barley soup using different concentrations of aqueous extract of saffron stigma during 12 days of storage at 30 °C

### Discussion

In this study, the inhibitory effect of the aqueous extract of saffron was evaluated on the growth of *S. Typhimurium*. It was found that extracts at concentration of 100 mg/ml could inhibit the growth of *S. Typhimurium* and this concentration was considered as MIC against this bacterium. It has been reported that ingredients, such as Crocin and Safranal are involved in this property. Probably due to the solubility in water, these compounds can bind to food-borne bacteria - and kill them (Pintado, 2011).

In line with the present study, Hashemi *et al.* evaluated the effect of aqueous extract of saffron stigmas on some pathogenic microorganisms. The MIC of aqueous extract in their study against all the studied bacteria was 40 mg/ml (Hashemi *et al.*, 2017). According to the results of the present study, the aqueous extract of saffron at a concentration of 100 mg/ml had an inhibitory effect against *S. Typhimurium*. In other similar studies, the antimicrobial effect of this extract on food-borne pathogens has also been proven (Cenci-Goga *et al.*, 2018, Jomehpour *et al.*, 2019, Pintado, 2011, Raj *et al.*, 2015, Razzaghi *et al.*, 2003). The results of a study performed on the aqueous extract of saffron stigmas collected from Torbat-e Heydarieh, Gonabad, and Khorasan (Iran) showed modest and obvious antibacterial activities against *Staphylococcus aureus* (*S. aureus*),

*Escherichia coli* (*E. coli*), and *Enterococcus faecalis* (Cenci-Goga *et al.*, 2018). Antibacterial activity of aqueous and methanolic extracts of *Crocus Sativus* stigma has also been reported against clinical isolates of some gram-positive and gram-negative pathogenic bacteria (Jomehpour *et al.*, 2019). Razzaghi *et al.* isolated the main components of aqueous extract and found that Safranal (an organic compound isolated from saffron) can inhibit the growth of *E. coli* and *S. aureus*. Other compounds of saffron had no inhibitory effects against the studied bacteria (Razzaghi *et al.*, 2003). In a study on the ethyl acetate, ethanol, petroleum ether extracts of stigma, cream, and saffron leaves on the *S. aureus*, *Staphylococcus epidermidis*, *E. coli*, *Micrococcus luteus*, *Candida albicans*, *Cladosporium*, and *Aspergillus niger*, it was shown that ethyl acetate extract of the saffron leaf did not affect the above microorganisms. The antifungal effect of the ethyl acetate extract of saffron stigma against the above microorganisms was more than style. The antibacterial activity of ethyl acetate extract of saffron style was more than other parts of the plant (Vahidi *et al.*, 2010). Muzaffar *et al.* also reported strong activity of the petroleum ether and methanolic extracts of saffron stigmas against various bacterial strains (*S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumonia*) and fungi (*Aspergillus*

*fumigates*, *Aspergillus niger*, and *Candida albicans*) (Muzaffar *et al.*, 2016). Antibacterial activity of the methanolic extract of stigma and petal of *Crocus spp.* (*Crocus caspius*, *Crocus speciosus* and *Crocus sativus*) has been reported against *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *E. coli*. The authors found that *Bacillus subtilis*, and *E. coli* were the most sensitive and resistant bacteria to the extract, respectively (Afshar Mohammadian *et al.*, 2016).

It has been reported that the aqueous extract of saffron has a great antimicrobial effect on coagulase-negative *staphylococci* (CoNs) (Ökmen *et al.*, 2016). Also, Fazeli Nasab *et al.* studied the antibacterial effects of hydroalcoholic extract of saffron petals on some gram-positive and gram-negative bacterial pathogens. They reported that the antimicrobial activity of this extract was more effective against gram-positive than gram-negative bacteria. The differences in the resistance of gram-negative bacteria to antimicrobial substances of the extract may be related to differences between bacteria in the cell wall structures. Generally, the cell wall of gram-positive bacteria consists of a single layer, whereas the gram-negative cell wall has a multilayered structure surrounded by an outer cell membrane (Fazeli Nasab, 2019, Gao *et al.*, 1999, Kim *et al.*, 2013).

In recent years, similar studies have been conducted to investigate the effect of plant essential oils and extracts against *S. Typhimurium* in food models. Moosavy *et al.* conducted a study about the effect of *Zataria multiflora* Bioss essential oil against *S. Typhimurium* and *S. aureus* in the commercial barley soup at 8 °C and 25 °C. They found that the increase of incubation temperature has a significant effect on the growth rate of this bacterium (Moosavy *et al.*, 2010). The results of the present study also indicated that the bacterial growth in commercial barley soup stored at 30 °C was more than those stored at 10 °C and 20 °C. Similar behavior has also been reported by other authors (McAuley *et al.*, 2015, Prudêncio *et al.*, 2015, Shakeri *et al.*, 2017, Tassou *et al.*, 2000). It may be related to the lower metabolic activity of *S. Typhimurium* at low temperatures (Shakeri *et*

*al.*, 2017, Tassou *et al.*, 2000). The temperature range for the growth of *S. Typhimurium* is from 6.2 °C to 45 °C. This microorganism can grow well at room temperature, but the optimum temperature for its growth is about 37 °C (Albrecht, 2021, Moosavy *et al.*, 2010). The low temperature had a bacteriostatic effect on *Salmonella spp.* allowing the antimicrobial to put more stress on the organism, resulting in the reduction of their count in food (Porter *et al.*, 2020). Moosavy *et al.* found that the count of *S. Typhimurium* in barley soup decreased by increasing the concentration of essential oil (Moosavy *et al.*, 2010). The results of the present study also showed that higher concentrations of saffron aqueous extract (200 mg/ml) increased the antibacterial effect of the saffron extract. Also, the results of this study are in agreement with the findings of other researchers. Moradi *et al.* studied the effect of *Cuminum cyminum* essential oil on *Bacillus cereus* in commercial barley soup, which was stored at 10 °C and 25 °C. They found that the count of bacteria at 10 °C was significantly lower than 25 °C (Moradi *et al.*, 2012). The results of Pajouhi *et al.* on the antimicrobial activity of *Origanum vulgare* and *Cuminum cyminum* essential oils in the barley soup showed that the use of these essential oils at low temperatures (8 °C) significantly inhibits the growth of the vegetative form of *Bacillus cereus* and *Bacillus subtilis* in commercial barley soup at the lowest concentration of each essential oil (Pajouhi *et al.*, 2012).

Generally, plant extracts and essential oils have been shown to prolong the delayed phase of bacterial growth while reducing the growth rate in the logarithm phase. Their performance follows a mechanism related to their accumulation in the lipid bilayer of the cell membrane and the destruction of its structure (Tassou and Nychas, 2000, Valero and Giner, 2006).

### Conclusion

The results of this study showed that the concentration of aqueous extract of saffron stigma and incubation temperature had a significant effect

on the behavior of *S. Typhimurium*. Therefore, high concentrations of this extract together with appropriate storage temperature can have an acceptable inhibitory effect on the growth of this bacterium, thereby increasing shelf life and improving food safety.

### Acknowledgment

The authors acknowledge the Vice Chancellor for Research and Technology of the University of Tabriz for their financial supports.

### Authors' Contribution

Valizadeh S: Data gathering and analysis, Project administration, Writing-original draft. Moosavy M: Conceptualization, Methodology, Supervision, Validation, Writing-original draft, Writing-review & editing. Ebrahimi A: Data analysis, Validation, Writing-original draft. Akhondzadeh Basti A: Conceptualization, Methodology, Supervision and, Validation. Mahmoudi R: Conceptualization, Data analysis, Methodology, Supervision, Validation. Khatibi SA: Software, Validation, Writing-original draft, Writing-review & editing.

### Conflicts of interest

The authors declare that there is no conflict of interest.

### References

**Afshar Mohammadian M, Kordi SH & Mashhadi Nejad A** 2016. Antibacterial activity of stigma and petal of different species of saffron (*Crocus* spp.). *Journal of Molecular and Cellular Research (Iranian Journal of Biology)*. **29** (3): 265-273.

**Ahmadi R, Alipour Eskandani M & Saadati D** 2017. Evaluation of antimicrobial effect of Iranian sumac on *Bacillus cereus* in a commercial barley soup. *Slovenian Veterinary Research*. **54** (2): 65-69.

**Albrecht JA** 2021. Salmonella. Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln, NE, USA.

**Amoozadeh A, Mohammadzadeh Milani J & Motamedzadegan A** 2016. Investigating the effect of brewing additives on the antioxidant activity of black tea. *Journal of food research*. **26** (3): 481-490.

**Cenci-Goga BT, et al.** 2018. In-vitro bactericidal activities of various extracts of saffron (*Crocus*

*sativus* L.) stigmas from Torbat-e Heydarieh, Gonabad and Khorasan, Iran. *Microbiology research*. **9** (1): 43-46.

**Dionisi G & Oldring PKT** 2002. Estimates of per capita exposure to substances migrating from canned foods and beverages. *Food Additives & Contaminants*. **19** (9): 891-903.

**Fazeli Nasab B** 2019. Evaluation of antibacterial activities of hydroalcoholic extract of saffron petals on some bacterial pathogens. *Journal of medical bacteriology*. **8** (5-6): 8-20.

**Gao Y, Van Belkum MJ & Stiles ME** 1999. The outer membrane of gram-negative bacteria inhibits antibacterial activity of brochocin-C. *Applied and environmental microbiology*. **65** (10): 4329-4333.

**Geiss HK, et al.** 1993. Food borne outbreak of a *Salmonella enteritidis* epidemic in a large pharmaceutical industry. *Gesundheitswesen* **55** (3): 130-135.

**Hashemi SM, Maassoumi SM & Gasempour HR** 2017. The antimicrobial properties of extracts in (*Crocus sativus* var. *haussknechtii* Boiss. and Reut. ex Maw). *Saffron agronomy and technology*. **5** (4): 407-412.

**Hedican E, et al.** 2009. Restaurant Salmonella Enteritidis outbreak associated with an asymptomatic infected food worker. *Journal of food protection*. **72** (11): 2332-2336.

**Hill T** 2004. The contemporary encyclopedia of herbs & spices: seasonings for the global kitchen. Wiley: New Jersey.

**Jomehpour N, Ghazvini K & Jomehpour M** 2019. Antibacterial activity of aqueous and methanolic extracts of *Crocus sativus* stigma and *Cinnamomum cassia* against clinical isolates of some gram-positive and gram-negative pathogenic bacteria. *Medical laboratory journal*. **13** (3): 31-34.

**Kalaivani R, Devi VJ, Umarani R, Periyanyagam K & Kumaraguru AK** 2012. Antimicrobial activity of some important medicinal plant oils against human pathogens. *Journal of biologically active products from nature*. **2** (1): 30-37.

**Karimi E, Oskoueian E, Hendra R & Jaafar HZ** 2010. Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. *Molecules (Basel, Switzerland)*. **15** (9): 6244-6256.

**Khatibi SA, Hamidi S & Siah-Shadbad MR** 2020a. Application of Liquid-Liquid Extraction for the Determination of Antibiotics in the Foodstuff: Recent

- Trends and Developments. *Critical reviews in analytical chemistry*. 1-16.
- Khatibi SA, Hamidi S & Siah-Shadbad MR** 2020b. Current trends in sample preparation by solid-phase extraction techniques for the determination of antibiotic residues in foodstuffs: a review. *Critical reviews in food science and nutrition*. **61 (20)**: 3361-3382.
- Khatibi SA, Misaghi A, Moosavy M-H, Amoabediny G & Akhondzadeh Basti A** 2015. Effect of preparation methods on the properties of *Zataria multiflora* Boiss. essential oil loaded nanoliposomes: characterization of size, encapsulation efficiency and stability. *Pharmaceutical science*. **20 (4)**: 141-148.
- Khatibi SA, et al.** 2017. Encapsulation of *Zataria multiflora* Boiss. essential oil into nanoliposomes and in vitro antibacterial activity against *Escherichia coli* O157:H7. *Journal of food processing and preservation*. **41 (3)**: e12955.
- Khatibi SA, et al.** 2018. Effect of nanoliposomes containing *Zataria multiflora* Boiss. essential oil on gene expression of Shiga toxin 2 in *Escherichia coli* O157:H7. *Journal of applied microbiology*. **124 (2)**: 389-397.
- Kim S-J, Cho AR & Han J** 2013. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food control*. **29 (1)**: 112-120.
- McAuley CM, et al.** 2015. *Salmonella* Typhimurium and *Salmonella* Sofia: growth in and persistence on eggs under production and retail conditions. *BioMed research international*. **2015**: 914987.
- Milajerdi A, Djafarian K & Hosseini B** 2016. The toxicity of saffron (*Crocus sativus* L.) and its constituents against normal and cancer cells. *Journal of nutrition & intermediary metabolism*. **3**: 23-32.
- Moosavy MH, et al.** 2010. Survey the effect of *Zataria multiflora* Boiss. essential oil on the growth of *Salmonella typhimurium* in a commercial barley soup. *Journal of medicinal plants* **9(34)**: 109-116.
- Moosavy MH, et al.** 2017. Antioxidant and antimicrobial activities of essential oil of lemon (*Citrus limon*) peel in vitro and in a food model. *Journal of food quality and hazards control*. **4 (2)**: 42-48.
- Moosavy MH, Shavisi N & Khatibi SA** 2018. Therapeutic effects of pumpkin in Islamic texts, Islamic Iranian traditional medicine and modern medicine. *History of medicine journal*. **9 (33)**: 77-92.
- Moradi B, Mashak Z, Akhondzadeh Basti A & Barin A** 2012. The survey of the effect of *Cuminum cyminum* L. essential oil on the growth of *Bacillus cereus* in a food model system. *Journal of medicinal plants*. **11 (41)**: 93-102.
- Moradi S & Sadeghi E** 2017. Study of the antimicrobial effects of essential oil of *Satureja edmondi* and nisin on *Staphylococcus aureus* in commercial soup. *Journal of food processing and preservation*. **41 (4)**: e13337.
- Muzaffar S, Rather SA & Khan KZ** 2016. In vitro bactericidal and fungicidal activities of various extracts of saffron (*Crocus sativus* L.) stigmas from Jammu & Kashmir, India. *Cogent food & agriculture*. **2 (1)**: 1158999.
- Mzabri I, Addi M & Berrichi A** 2019. Traditional and Modern Uses of Saffron (*Crocus sativus*). *Cosmetics*. **6 (4)**: 63.
- Ökmen G, Kardas S, Bayrak D, Arslan A & Çakar H** 2016. The antibacterial activities of *Crocus sativus* against mastitis pathogens and its antioxidant activities. *World journal of pharmacy and pharmaceutical sciences*. **5 (3)**: 146-156.
- Oussalah M, Caillet S, Saucier L & Lacroix M** 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food control*. **18 (5)**: 414-420.
- Pajohi M, Tajik H & Farshid A** 2010. Antimicrobial activity of Nisin on *Bacillus cereus* and *Bacillus subtilis* in a food model and their ultra structural investigation. *Iranian journal medical microbiology*. **4 (3)**: 45-52.
- Pajouhi MR, Tajik H, Akhondzadeh Basti A, Gandomi H & Ehsani A** 2012. A study on chemical composition and antimicrobial activity of essential oil of *Mentha longifolia* L. and *Cuminum cyminum* L. in soup. *Iranian journal of food science and technology*. **9 (36)**: 33-45.
- Pandey AK, Kumar P, Singh P, Tripathi NN & Bajpai VK** 2017. Essential Oils: Sources of Antimicrobials and Food Preservatives. *Frontiers in microbiology*. **7**: 2161.
- Parray JA, Kamili AN, Hamid R, Reshi ZA & Qadri RA** 2015. Antibacterial and antioxidant activity of methanol extracts of *Crocus sativus* L. c.v. Kashmirianus. *Frontiers in life science*. **8 (1)**: 40-46.



- Pintado C** 2011. Bactericidal effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* during storage. *Food control*. **22** (3-4): 638-642.
- Pitsikas N & Sakellaridis N** 2006. *Crocus sativus* L. extracts antagonize memory impairments in different behavioural tasks in the rat. *Behavioural brain research*. **173** (1): 112-115.
- Porter JA, Morey A & Monu EA** 2020. Antimicrobial efficacy of white mustard essential oil and carvacrol against *Salmonella* in refrigerated ground chicken. *Poultry science*. **99** (10): 5091-5095.
- Prudêncio CV, Mantovani HC, Cecon PR & Vanetti MCD** 2015. Differences in the antibacterial activity of nisin and bovicin HC5 against *Salmonella* Typhimurium under different temperature and pH conditions. *Journal of applied microbiology*. **118** (1): 18-26.
- Raj P, et al.** 2015. Biodiversity of endophytic fungi in saffron (*Crocus sativus*) and antimicrobial activity of their crude extract. *Indo American journal of pharmaceutical research*. **3**: 3702-3713.
- Razzaghi R, Noorbakhsh R, Kakhaki A & Najafi M** 2003. Evaluation of antimicrobial effects of *Crocus sativus* stigmas constituents. In *Proceedings of the third national symposium on saffron*, pp. 239-244: Mashad, Iran.
- Santos-Sánchez NF, Salas-Coronado R, Valadez-Blanco R, Hernández-Carlos B & Guadarrama-Mendoza PC** 2017. Natural antioxidant extracts as food preservatives. *Acta Sci Pol Technol Aliment*. **16** (4): 361-370.
- Shahbazi Y, Shavisi N & Mohebi E** 2017. Antibacterial activity of *Ziziphora clinopodioides* essential oil and nisin against *Bacillus subtilis* and *Salmonella* Typhimurium in commercial barley soup. *Bulgarian journal of veterinary medicine*. **20** (1): 65-72.
- Shakeri G, Jamshidi A, Khanzadi S & Azizzadeh M** 2017. Modeling of *Salmonella typhimurium* growth under the effects of *Carum copticum* essential oil, temperature, pH and inoculum size. *Veterinary research forum*. **8** (1): 59-65.
- Sharafati Chaleshtori R & Fallah M** 2019. Antibacterial effect of *Bunium persicum*, *Eucalyptus globules*, and *Allium ampeloprasum* extracts on STEC and MRSA in commercial barley soup. *Advanced herbal medicine*. **5** (2): 9-20.
- Tassou C, Koutsoumanis K & Nychas GJE** 2000. Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food research international*. **33** (3): 273-280.
- Tassou C & Nychas G-J** 2000. Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food research international*. **33**: 273-280.
- Vahidi H, Kamalinejad M & Sedaghati N** 2010. Antimicrobial Properties of *Crocus Sativus* L. *Iranian journal of pharmaceutical research*. **1** (1): 33-35.
- Valero M & Giner MJ** 2006. Effects of antimicrobial components of essential oils on growth of *Bacillus cereus* INRA L2104 in and the sensory qualities of carrot broth. *International journal of food microbiology*. **106** (1): 90-94.
- Vo TH, Le NH, Cao TTD, Nuorti JP & Minh NNT** 2014. An outbreak of food-borne salmonellosis linked to a bread takeaway shop in Ben Tre City, Vietnam. *International journal of infectious diseases*. **26**: 128-131.
- Wadhvani T, et al.** 2009. Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. *Internet journal of microbiology*. **7** (1): 1-14.