Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2022; 7(2): 160-166 Website: jnfs.ssu.ac.ir

Study of Antioxidant Effects of Alcoholic Extract of Mentha longifolia on Fat Oxidation of High-Consumption Meat Products in Tabriz

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ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 7 Apr 2021
Revised: 3 Jul 2021
Accepted: 13 Sep 2021

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ABSTRACT

Background: Fat peroxidation is one of the problems in food processing, cooking, and storage. Oxidative changes cause pathological effects in biological systems and reduce the taste and quality and ultimately spoil food. Using aromatic plants and vegetables with antioxidant properties can play an important role in preventing these adverse effects. The antioxidant effects of Mentha longifolia have been considered in recent years.This study aimed to investigate the antioxidant activity of the peppermint plants and determine its protective effect on lipid oxidation of high-consumption meat products in Tabriz. **Methods:** To this end, aqueous-alcoholic extract of mint leaves and twigs with concentrations of 0.1%, 1%, 5% with homogeneous mixtures of 10%, 90% meat hamburger products, and 45% sausages were exposed. Lipid oxidation was measured by measuring malondialdehyde using the TBARS method on day 0 and after 42 days of exposure to peppermint extract at 0-4 ℃ and was compared with the control group. **Results:** The results showed that increasing the shelf life of meat mixtures significantly increases fat peroxidation (P = 0.05). Also, increasing the concentration of peppermint extract in meat solution samples significantly reduces the amount of malondialdehyde (P = 0.05). The results showed the antioxidant activity of peppermint during the storage time of meat products and its benefits can be used in the food industry. **Conclusion:** The present study showed that peppermint extracts have strong antioxidant activity and can be used in food and biological systems and can be used as a substitute for synthetic antioxidants to reduce their harmful effects on health.

This paper should be cited as: Hejazy M, Abdollahzadeh M, Javid F. ***Study of Antioxidant Effects of Alcoholic Extract of Mentha Longifolia on Fat Oxidation of High-Consumption Meat Products in Tabriz.*** *Journal of Nutrition and Food Security (JNFS)*, 2022; 7 (2): 160-166.

[DOI: 10.18502/jnfs.v7i2.9329]

Keywords: *Mint; Antioxidant; Lipid oxidation; Meat products*

Introduction

Medicinal plants, their extracts, and even vegetable oils play an important role in the health of the human body. In recent years, due attention has been paid to the content and antioxidant activity of medicinal plants, vegetables, fruits, and their effect in reducing the degradation of biochemical molecules (Paymard *et al.*, 2013).

Studies have shown that free radicals are one of the most important oxidizing agents in food. These oxidative changes will eventually lead to a decrease in the quality level of the food, its odor, and bad taste (Kang *et al*., 2006).

Free radicals are active molecules and are naturally produced in the human body during the process of metabolism. Free radicals may also be produced in diseases, such as diabetes, which will be eliminated from the body if combined with antioxidants (Kohen *et al.*, 1996). Important antioxidants include superoxide dismutase, glutamine peroxidase, and catalase. The damaging effects of free radicals on fat, protein, carbohydrate, and DNA have been reported. The oxidative process of fats is the result of combining fats with free radicals; the destructive effect of this process is naturally neutralized as a result of the activity of the antioxidant defense system (Halliwell and Gutteridge, 2015, Halliwell *et al.*, 1997). The process of hydrolysis in food, especially in meat products that occurs in the presence of free radicals, leads to the production of an aldehyde called malondialdehyde (MDA), which is the main cause of bad taste and smell of meat (Guillen and Cabo, 1997).

Lipid peroxidation is a process that occurs as a result of increasing the production of free radicals or decreasing the body's antioxidant levels. Under these conditions, the cellular structure of fatty acids with several double bonds, such as EPA (Eicosapentaenoic) and DHA (Decosahexaenoic), are present in cell membrane. If this process continues, the oxidative degradation of MDA will be produced. This event eventually leads to cell death (Baynes, 1991, Sant'Ana and Mancini-Filho, 2000). The use of natural antioxidants can reduce the rate of food oxidation and if properly used and applied can increase the life of food products during consumption (Bouayed, 2010).

Studies have shown that there is a direct relationship between the presence of phenolic compounds and antioxidant properties. The higher the amount of phenolic compounds in plant extracts, the higher the antioxidant properties (Bahramikia and Yazdanparast, 2008). Also, the effective role of phenolic compounds as eliminators of free radicals has been reported in several studies (Velioglu *et al.*, 1998).

Phenolic compounds, due to their benzene ring and electron resonance properties, trap free radicals and prevent the development of chain reactions and the production of free radicals (Khosravinia *et al.*, 2015). Moreover, according to studies by Ilalyaraja *et al.*, phenolic compounds in plants and fruits are considered valuable antioxidants due to their reducing role (hydrogen ion donor) and metal abduction (Ilaiyaraja *et al.*, 2010).

The use of flavonoids and other phenolic compounds in food is recommended to reduce the incidence of cardiovascular disease and cancer (Shahidi and Ambigaipalan, 2015). Studies have shown that the antioxidant activity in plant extracts and some fruits due to the presence of flavonoids, carotenoids, and ascorbic acid (Gorinstein *et al.*, 2004). Antioxidant micronutrients, such as vitamins E and C and carotenoids have the ability to remove

[DOI: 10.18502/jnfs.v7i2.9329]

active oxidants in the body (Ozsoy *et al.*, 2009). Also flavonoids have more antioxidant activity to eliminate free radicals (Pourmoghim *et al.*, 2015). In the structure of some non-volatile components of alcoholic extracts of fruit skins, flavonoid and phenolic antioxidant compounds have been identified (Guo *et al.*, 1997). According to research results, antioxidant capacity decreases over time due to reduced amounts of polyphenols and vitamin C (Klimczak *et al.*, 2007). Due to the toxicity and carcinogenicity of synthetic and synthetic antioxidants, such as butyl hydroxytoluene (BHT) and butyl hydroxy anisole (BHA), today researchers have identified natural antioxidants (Ito *et al.*, 1983, Li *et al.*, 2006).

This study aimed to investigate the protective effect of Iranian peppermint extract on lipid oxidation of high-consumption meat products in Tabriz, including hamburgers and raw sausages.

Materials and Methods

Preparation of extracts: 40 g of mint powder was mixed in 320 ml of 72% ethanol. It was then placed in a 40 ℃ pan for 3 hours. The suspension was poured into test tubes and centrifuged at 3000 rpm for 10 min. The surface liquid was then filtered with Whatman 42 filter paper. The remaining pulp was also re-extracted. Then, in order to concentrate the extracts, the filtered solutions were concentrated in a rotary evaporator. The concentrate was dried in an incubator at 27 ℃. The final material was stored in the freezer at -20 ℃ until it is ready to be used.

Preparation of test samples: Four samples of 45% sausages and 4 samples of hamburgers 90% of the daily production samples were purchased in Tabriz. The samples were completely crushed and homogeneous. Then 10% aqueous solutions were prepared from each sample of meat product. Then 4 ml of the prepared suspension was removed and placed in a test tube. Then, 500 µl of prepared peppermint extracts with concentrations of 0.1%, 1%, and 5% were added to the test tubes. After that, 500 µl of distilled water was added to the negative control tube and 0.01% ethanol of BHT solution was added to the positive control tube of 500 µl.

MDA measurements by TBARS method (short and after 30 min): According to the method of Ahn et al. (Ahn *et al.*, 1999), to measure MDA on day 0 of the experiment, all test tubes were placed in a boiling pan for 15 min. The contents of the test tubes were then shaken using a shaker for 15 min. They were then boiled again for 40 min. After 30 min, the TBARS values were measured. These measurements were performed with three replications.

Measurement of MDA by long-term TBARS method (after 42 days): According to the method of Moller et al. (Møller *et al.*, 1999), all test tubes were stored for up to 42 days in the dark and refrigerated at 4 ℃.

After leaving the refrigerator and reaching the temperature of the tubes at laboratory temperature, 2 ml of TBA solution (57.66 mg of TBA in glacial acetic acid and 5 µl of 0.01% ethanol BHT solution was added to all tubes. They were shaken for 20 min in a shaker and then boiled in a boiling pan for 40 min, and finally their MDA values were measured by TBARS method, all of which were repeated three times. Changes in the amount of MDA were read at 532 nm in front of the control using a light absorption spectrophotometer.

Results

Short-term TBARS assessment: Values in meat samples containing hamburgers 90%, concentrations of 0.1%, 1%, and 5% of plant extracts, the amount of MDA in 30 min (on day 0 of the experiment) decreased compared to the control and the highest reduction was 5% of mint extract. However, these reductions were not significant during this period.

TBARS at zero test time in non-extracted test tube, test tube containing 0.1%, 1%, and 5% extract was 1.78, 1.73, 04, and 1.611 micromolar, respectively, which after half an hour were 1.92, 2.03, 77.1 and 1.1 micromolar, respectively (**Figure 1**).

In the case of meat samples containing 45% sausages in the non-extracted test tube, the test tube containing 0.1%, 1%, 5% was 64.1, 61.1, and 50.1, 28.1 µM, respectively results were 88.1, 82.1, and 63.41,1.1 µM after half an hour (**Figure 2**).

[DOI: 10.18502/jnfs.v7i2.9329]

Long-term TBARS assessment: The results showed that increasing the concentration of the extract has inhibitory effects on the lipid oxidation process significantly ($P \leq 0.05$) in both types of meat products. The alcoholic solution of the extract with a concentration of 5% showed the lowest amounts of TBARS in raw hamburger meat 90% and sausage 45%. In the raw hamburger sample, 90% had no extract at the time of zero 1.78 µM, which increased to 4.173 µM after 42 days. While in the sample containing 5% alcoholic solution of peppermint extract, this value increased from 04.1 µM at zero time to 3.007 µM in 42 days (**Figure 1**).

In the sample of 45% raw sausage without extract, at zero time, the amount of TBARS was 1.64 µM, which increased to 127.4 µM after 42 days. While in the sample containing 5% alcoholic solution of peppermint extract, this value increased from 28.1 µM at zero time to 111.3 µM in 42 days (**Figure 2**).

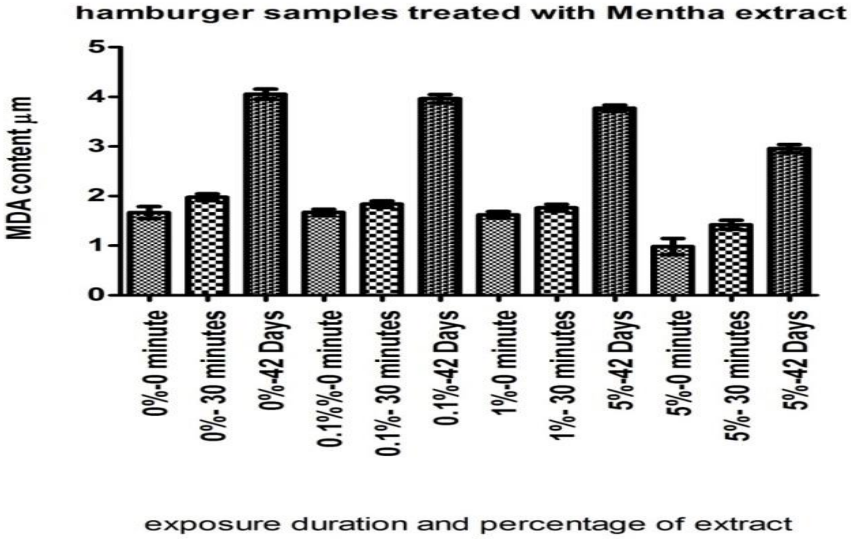
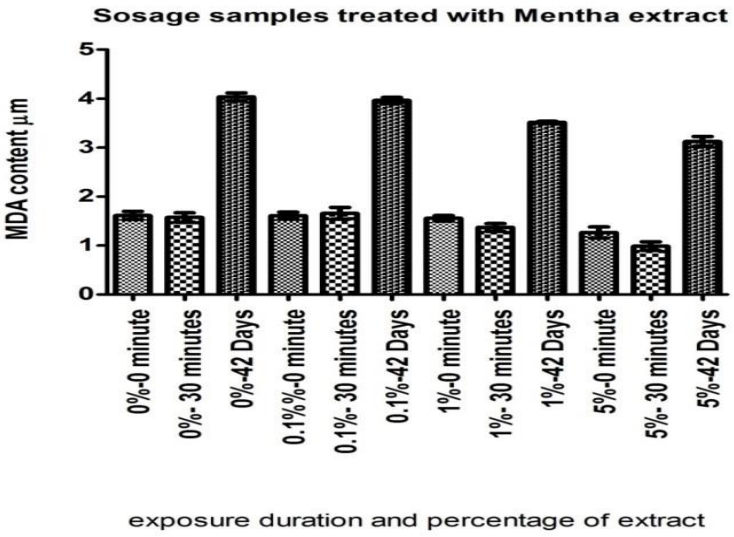


Figure 1. Malondialdehyde content in hamburger samples at 0, 30 min, and 42 days with different percentages of peppermint extract



[DOI: 10.18502/jnfs.v7i2.9329]

Figure 2. Malondialdehyde content in sausage samples at 0, 30 min, and 42 days with different percentages of peppermint extract

The results of TBARS test indicate the presence of antioxidant activity of peppermint extract and its stability over more than one month. All stages of the test were along with some changes according to the source (Zahra *et al.*, 2010).

Discussion

In the raw meat hamburger sample without peppermint extract, the average amount of MDA at time zero was 1.78 µM and increased to 4.173 µM after 42 days. While this amount in meat samples containing 5% extract at zero time was from 1.04 micromolar to 3.007 micromolar, after 42 days. In similar samples of 45% sausages, the average amount of MDA in the meat-free solution at the time of zero was 64.1, which reached 4.127 µM after 42 days. The meat solution contained 5% of the extract at 28.1 µM at zero time, which reached 11.13 µM after 42 days. The antioxidant effect depends on several factors, such as environment, temperature, type of substrate, oxidation conditions, and physical condition of the oxidation environment (Laguerre *et al.*, 2007). Antioxidant activity of plants and fruits can be affected by the geographical conditions of the region, type of agricultural soils, planting and harvesting conditions as well as storage time of that product (van der Sluis *et al.*, 2001). Plants that are rich in phenolic compounds, such as flavonoids and flavonols have higher antioxidant power (Seifollah *et al.*, 2012).The results of short-term TBARS test showed that the effect of the extract was directly and significantly related to its concentration. So that the higher the concentration of the extract, the greater the effect in preventing lipid oxidation and increasing the amount of MDA. Studies on long-term TBARS experiments have shown that by increasing the concentration of the extract, the rate of inhibition of lipid oxidation increases and it was found that the 5% extract is significantly more capable of preventing lipid oxidation and increasing the amount of MDA in products. It has hamburger and sausage meat. It was also found that lipid oxidation increased in these meat products over time. In the present study, TBARS levels in hamburger and raw sausage samples increased over time. These results were consistent with the findings of the study by Fasseas *et al.* (Fasseas *et al.*, 2008). Due to hydrolysis and lipid oxidation, hydroperoxides are produced in these meat samples during product storage. It will also provide more opportunities to produce lipid oxidation secondary reaction products that react with TBA (Chaijan *et al.*, 2006). Increased lipid oxidation over time can be due to the release of peroxidants due to excessive decomposition during storage (Chaijan *et al.*, 2005). According to Weber, the cooking method is also effective in TBARS. Boiling increases the amount of TBARS, but grilling does not change it (Weber *et al.*, 2008). Comparison of changes in MDA values in cooked and raw meat samples shows that cooking itself stimulates the process of fat oxidation and the amount of lipid oxidation in cooked meat has been reported to be higher than raw meat (Widayaka *et al.*, 2001). A study on fish meat showed that lipid oxidation in fish meat is affected by internal and external factors, such as fatty acid composition, concentration of peroxidants, enzymes, temperature, pH, oxygen, and ionic interactions (Renerre and Labas, 1987). It is mentioned that in fish meat, oxidation and hydrolysis processes both change the color of the meat and create a bad taste and smell, and ultimately lead to spoilage of the food (Pacheco- Aguilar *et al.*, 2000).

Conclusion

The present study showed that peppermint extracts have strong antioxidant activity and can be used in food and biological systems and can be used as a substitute for synthetic antioxidants to reduce their harmful effects on health. For this purpose, Iranian peppermint extract needs further research and identification of its most effective components.

[DOI: 10.18502/jnfs.v7i2.9329]

Acknowledgment

This research did not receive any specific grant support from funding agencies in the public, commercial, or not-forprofit sectors.

Conflict of interest

The authors declare no conflict of interests.

Authors' contributions

Hejazy M developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and is guarantor. Abdollahzadeh M and Javid F contributed to the development of the protocol, abstracted data, and prepared the manuscript.

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[DOI: 10.18502/jnfs.v7i2.9329]