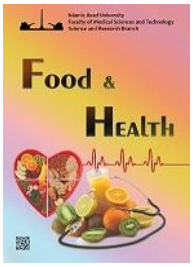

Food & Health Journal
Journal homepage: fh.srbiau.ac.ir

The effect of aqueous and ethanolic extract of Hollyhock black (*Alcea rosea*) on physicochemical and antioxidant properties of ketchup sauce

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ARTICLE INFO

Original Article

Article history:
Received 20 April 2021
Revised 12 July 2021
Accepted 11 August 2021
Available online 20 September 2021

Keywords:
Alcea rosea
Essential oil
Ketchup
Natural antioxidant
Vitamin C

ABSTRACT

The aim of this study was to investigate the effect of *Alcea rosea* extracts on the physicochemical, antioxidant properties of ketchup sauce. To achieve this goal, aqueous and ethanolic extracts, separately, were added to the ketchup formulation at three levels of 0.2, 0.5, and 0.8%, as well as some traits of the samples, were tested. The results showed that the acidity was ranged from 2.3-3.2.40%. The different levels of extracts had not a significant effect on the acidity, brightness, and TSS. The total phenol content, followed by a significant enhancement in antioxidant activity and retention of vitamin C ($p<0.05$). The total phenol, vitamin C, and antioxidant activity of samples containing aqueous extract were significantly higher than ethanolic extract. The results of the sensory evaluation showed that samples containing 0.2% and 0.5% aqueous extract had similar sensory acceptance to the control. Taste and odor scores of other treatments were not different from the control, but their color and overall acceptance scores were significantly lower than the control. Based on the obtained results, it can be concluded that extracts, (especially aqueous extract), have significant pragmatic activity and can be used as natural antioxidants to better preserve vitamin C in ketchup sauce.

1. Introduction

Ketchup is a popular food that has recently received attention due to its lycopene content and health-promoting features. It is produced in different countries with different sensory quality and nutrition (1). Tomato ketchup is a flavored product that contains a variety of ingredients that are made either from fresh tomatoes or from concentrates such as tomato puree and paste with sweeteners, salt, vinegar, and spices (2). Today, due to the unhealthy effects of chemical and synthetic preservatives, consumers want to use natural preservatives derived from plant, animal, and microbial sources, in addition, to protecting themselves from the harmful impacts of chemical preservatives by increasing the shelf life of food. Essential oils are aromatic liquids obtained from various components of the plant. The quantity and quality of chemical compounds of essential oil can vary depending on plant type, climate, soil composition, and plant age. The bioactivity properties of essential oils are generally determined by the major compounds present in them. The most common use of these substances is in EU member states. These include the cosmetics, health, and perfume industries, medical and pharmaceutical parts as antimicrobials, and improvement of the taste of medicines. Aromatic oils are generally colorless, especially when freshly prepared, but over time, their color darkens due to oxidation and resin. In order to prevent such changes, it should be stored in a dry, cool place and closed and dark glass containers. A variety of plants have been identified that contain many types of immunomodulatory polysaccharides. Studies show that most of these compounds, due to the activation of the host immune system, have more antitumor and antimicrobial properties than toxic effects (3, 4). *Alcea rosea*, the common hollyhock, is an ornamental dicot flowering plant in the family *Malvaceae*. It was imported into Europe from southwestern China during, or possibly before, the 15th century. In herbal medicine, hollyhock is believed to be an emollient and laxative. It is used to control inflammation, to stop bedwetting, and as a mouthwash in cases of bleeding gums. A search through the literature stated that the antibacterial effect of ethanolic extract of marshmallow root

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had antibacterial activity and the growth inhibition of the extract had a logarithmic relationship with increasing concentration (5). Elmastas *et al*. (6) evaluated the antioxidant activity of marshmallow flowers using various tests including, free radical and metal scavenging activity. The results of (7) generally marked that the darker the color of marshmallow flowers shows the effective antioxidant activity. Habibian *et al*. (8) revealed that *Althaea officinalis* aqueous extracts have antifungal effects against *Penicillium spp* in all applied concentrations. The study of Babolani Mogadam *et al*. (9) confirmed that the use of antimicrobial films incorporated with ajwain essential oil has a remarkable antibacterial effect in the food industry. During refrigerated storage sausage in terms of color parameters and free radical scavenging activity, the treatments with *S. leriifolia* extracts were better than control and treatments containing *Z. multiflora* extracts, while, the physicochemical and sensorial properties of recent treatments were better than *S. leriifolia* extracts and control (10). Investigation of the effects of antimicrobial alone and in combination with other factors (such as temperature, etc.) in the growth of bacterial pathogens transmitted by food and spoilage microorganisms, is necessary for laboratory and food models for application of the essential oils as preservatives in food. Due to the identification of antimicrobial side effects and the development of their-resistant fungi, there is growing interest to replace antimicrobial with herbal remedies. The present study was conducted to evaluate the antifungal effects of *Alcea rosea* aqueous/ethanolic extracts on ketchup sauce.

2. Materials and methods

The dried flowers of the plant were pulverized by the electric mill and 20 g of the plant powder was poured separately into Erlenmeyer flasks containing 400 ml of water and 80% ethanol. After 48 hours of incubation at 50 ° C, the samples were passed through Whatman filter paper. Water/ethanol was added to the resulting sample and the previous steps were repeated. Then the extracts were separated from the solvent and concentrated and their volume was increased to 20 ml, using a rotary evaporator at 50 ° C (5). Tomato paste (20%), sugar (13%), vinegar (10.5%), liquid glucose equivalent to dextrose 42 (8.2%), salt (1.6%), modified starch corn (2%), guar gum (0.2%), spices including onion and thyme powder (0.4%) and water (47.1%) were used to prepare ketchup. Finally, it was heated at 90 ° C for 2 minutes and the ketchup sauce was poured into special containers and packed. Aqueous and alcoholic extracts of plants were added to ketchup formulation at the levels of 0.2, 0.5, and 0.8%. The amount of soluble solids was calculated by a digital refractometer (11). The acidity was measured by titration method with 0.1 normal NaOH, and was stated in terms of percentage of citric acid (7). The level of vitamin C was recorded by the method of Mashayekhi and Atashi (12) as a two-stage oxidation-reduction titration. The phenol content was determined according to (13) with some modifications. Briefly, 300 µL of appropriately diluted bitter melon extract or standard solution was mixed and incubated with 300 µL of FC solution for 2 min before 2400 µL of a 50 g/L sodium carbonate solution was added. The solutions were mixed well and placed in the dark at room temperature for 2 h before the absorption was measured at 765 nm using a spectrophotometer. Gallic acid was used as the standard and the phenol content was expressed as mg gallic acid equivalents per g of dry powder weight (mg GAE/g). DPPH values are calculated according to (14). Color indices of ketchup sauce samples including light intensity (L^*), red-green intensity (a^*), and yellow-blue intensity (b^*) were determined using a Hunterlub device. The sensory characteristics of ketchup sauce including color, taste, smell, and general acceptance were expressed by 10 trained evaluators from 1 to 5 (based on a five-point hedonic test) (15). The data were analyzed by SPSS 19 statistical software and the comparison of means was evaluated using Duncan's Multiple Range Test at the level of 1% and 5%. Excel software was also used to draw the graphs.

3. Results and discussion

A large number of essential oils are able to reduce the number of bacteria. For example, the majority of essential oils tested had a significant inhibitory effect on pathogenic microorganisms. The antibacterial effect of essential oils on food pathogenic bacteria has been evaluated and it has been found that experimental inoculation of essential oils increases the shelf life of these products. By controlling the growth of bacteria, undesirable changes in the organoleptic properties of food products are reduced. Molds and yeasts can grow in most foods and produce compounds based on flavors and toxins, and cause discoloration and proteolysis due to the activity of various enzymes such as lipases and proteases. Fortunately, plant-based essential oils also have antifungal properties. Essential oils can make a difference in the aroma, taste, and other characteristics of food products. Plant essential oils cause granulation of the cytoplasm and organs of the cytoplasmic membrane and inactivation of intracellular and extracellular enzymes that affect the growth of microorganisms and cause their disintegration and inactivation. Based on studies it has been shown that compounds increase membrane permeability and penetrate the membrane, causing the membrane to swell and affect its function (16). As shown, the effect of a kind and concentration extraction on vitamin C, phenol, antioxidant activity, a, b color indices, and acceptance were significant at the 1% level. There is a significant statistical interaction kind and concentration in vitamin C, phenol, antioxidant activity, and a color index. Our results clearly showed that plant extraction influenced the vitamin C, phenol as well as antioxidant characteristics of ketchup throughout treatment (Fig. 1, 2, 3). Based on our research, increasing the level of aqueous and ethanolic extracts in ketchup sauce formulation caused a significant increase in vitamin C and phenol content. Vitamin C is a highly sensitive water-soluble material that is rapidly degraded by oxidation reactions in the presence of heat and oxygen. Its reduction occurs mainly by chemical degradation and conversion of ascorbic acid to

oxidation to dehydroascorbic acid, followed by hydrolysis of 3, 2-dictoglonic acid and polymerization into inactivated (nutritionally) products (17). The content of phenolic compounds in products is directly related to the retention of vitamin C in them.

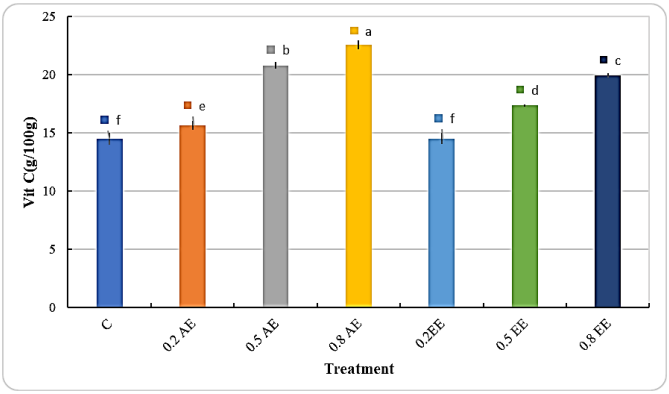

Fig.1. Changes of vitamin C in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %).

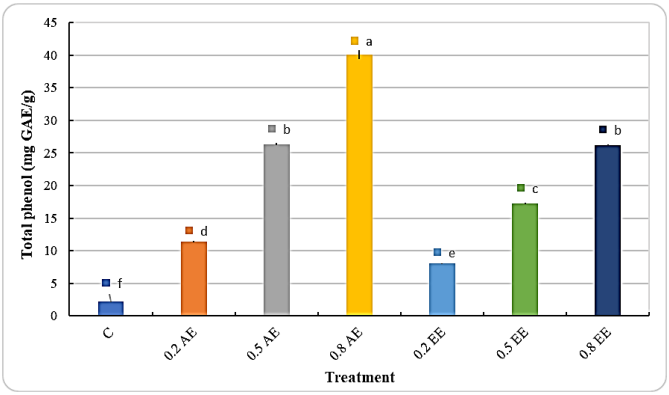

Fig. 2. Changes of total phenol in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %)

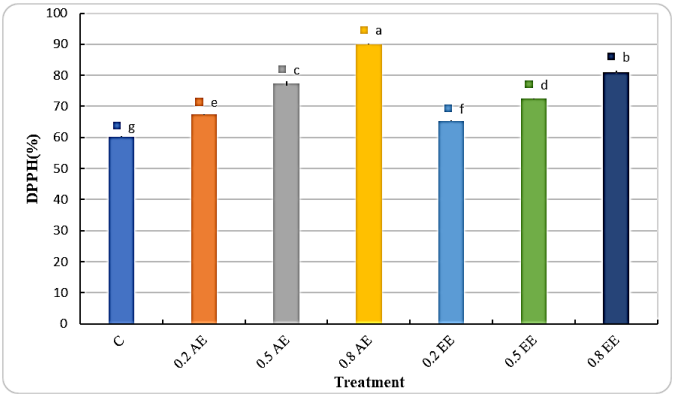

Fig.3. Changes of DPPH in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %).

Increasing the concentration of phenolic compounds enhanced the ability to inhibit free radicals. At higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups in the environment, the probability of donating hydrogen to the free radical increases and leads to an increase in the inhibitory vigor of the extract, which delays the oxidation reaction and subsequent survival of vitamin C. (18). Therefore, in the present study, due to the higher antioxidant activity of aqueous extract compared to its ethanolic extract, ketchup sauce samples containing aqueous extracts had higher vitamin C content than samples of alcoholic extract. Noktesanj avalet al. (19) reported a significant increase in vitamin C content of tomato sauce due to adding olive leaf extract. Two-component solvent systems are more desirable in extracting phenolic compounds than single-component ones. In a study by Fernandez *et al*. (20), the highest phenol content was obtained from ethanol/ methanol extract, and the lowest was extracted with water, which was not consistent with our study. Dehghani *et al*. (15) reported an increase in the total phenolic content of ketchup due to the addition of walnut green skin extract. Our experience illustrated that since the control sample had the lowest amount of phenolic compounds; it also showed the minimum percentage of free radical scavenging. Pouladi et al. (21) evaluated the DPPH method that the methanolic extract has the ability to inhibit or neutralize DPPH radicals. In (22) study, Velik green leaf extract has a significant amount of phenolic compounds that inhibit free radical DPPH and consequently has antioxidant and chelating properties and its use in soybean oil causes a significant reduction in acid and peroxide indices. Acidity is one of the important factors in the quality of ketchup sauce because it affects the thermal process conditions required to produce safe products. It is possible that the sauce acidity does not allow microbial growth. Therefore, the acidic nature of the sauce improves the ability of the essential oil to penetrate the bacterial cell membrane. Tomato products are in the category of acidic and their pH is often less than 4.5, so they need milder processing conditions to control microbial spoilage and inactivation of enzymes (23). National Standard of Iran (24), No. 2550 has determined the maximum acidity of tomato sauce 2.5% in terms of acetic acid and the results of this study showed that the acidity of different samples of ketchup sauce in the range of 2-40 (Fig. 4). It was 2.33 %, which corresponded to the values set by the Iranian standard. In previous scientific studies, various additives were used in ketchup sauce formulations and different results were reported. Amini et al. (11) stated that the acidity of ketchup sauce was 1.65%, which was lower than the acidity of ketchup produced in our study. The pH and acidity results were consistent with the results of (25). It can also be said that ketchup sauce has not been a suitable environment for the survival of these bacteria due to the low pH of free water restriction, the use of hydrocolloid compounds in the formulation, and the application of the thermal process. In addition, researches showed that the common salt can intensify

the antimicrobial effects of plant essential oils. Azeri Joghan *et al.* (26) investigated the antimicrobial effect of thyme and rosemary essential oil in ketchup sauce, the pH and microbial count were decreased when the acidity was increased. Similar to our results, it has been reported that marshmallow extract has antimicrobial, especially antifungal effects.

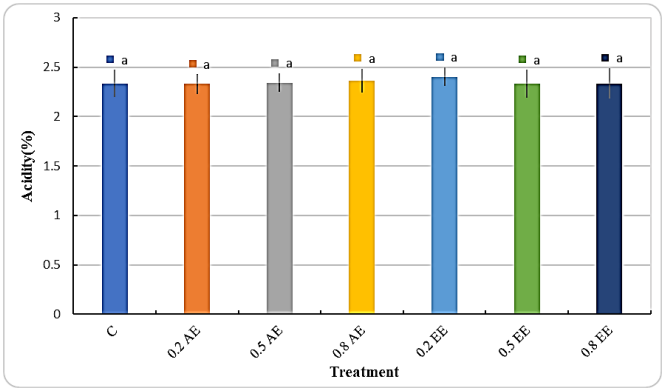

Fig.4. Changes of acidity in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %).

It was expressed alcoholic extract of the plant contains a compound of saponin which has been antibacterial properties (27). The results of our study showed that the addition of different levels of hollyhock black extracts had no significant effect on the Brix degree of the samples (Fig. 5).

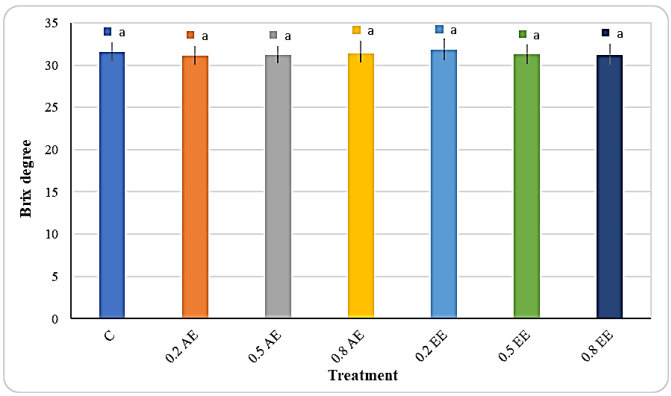

Fig. 5. Changes of brix degree in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %).

According to Iranian National Standard No. 2550, the minimum amount of soluble solids in tomato sauce is 30 ° Brix. Our results indicated that the ° Brix ranges from 31.15-31.85, which corresponded to the values set by the Iranian standard (24). Mesbahi et al. (16) observed that adding different levels of the skin and tomato seeds to ketchup sauce significantly increased the Brix of the samples. Mohamed *et al.* (28) pointed out that Sumac aqueous extract did not cause a significant change in the Brix of the sauce samples. The application of extracts did not have a significant effect on the color brightness of ketchup sauce samples, but reduced the intensity of yellowing and increased the intensity of redness (excluding 0.2 and 0.5% levels of aqueous extract) (Fig. 6).

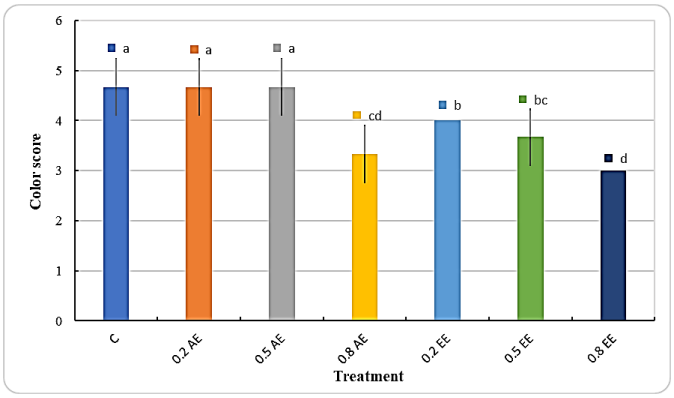

Fig. 6. Changes of color score in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %) score changed to score in y-axis.

Improving the red color of ketchup by adding hollyhock black extracts, especially aqueous extract, is probably related to their significant antioxidant activity, which has resulted in better preservation of lycopene pigment in ketchup samples. In another study, the colorimetric test revealed that in the mayonnaise samples containing savory, the tendency to redness was low, but the tendency to jaundice increased. Also, in the samples consisting of preservatives, the amount of transparency and clarity was higher than the samples with savory (29). In the study of Farahnaky *et al.* (30) had significantly higher color brightness (50-56-84) than the ketchup produced in the present study. The difference in the amount of color characteristics of production sauces in different studies is due to their various formulations, production methods, and properties of raw materials. The addition of 0.2 and 0.5% levels of aqueous extract did not have a negative effect on the color and overall acceptance of ketchup sauce samples, but other treatments had lower color scores and acceptance than the control.

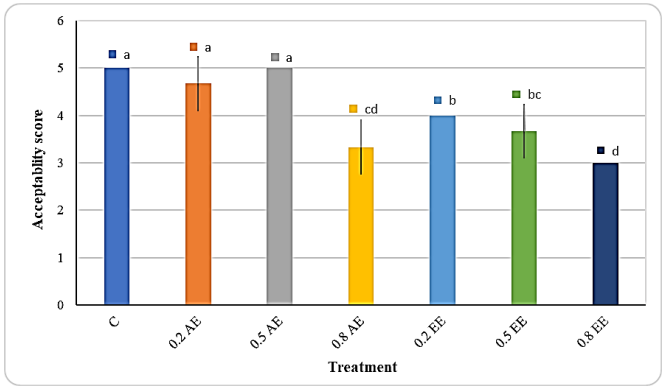

Fig.7. Changes of overall acceptability score in ketchup sauce treated with aquatic extraction (AE) and ethanolic (EE) extraction (0, 0.2. 0.5 and 0.8 %).

However, according to the results of sensory evaluation, it can be said that the level of acceptance of sauce containing essential oils has been relatively good, but not ideal (Fig. 7). It seems that people are not familiar with the taste of herbs and medicinal plants and the great popularity of this type of sauce is the main reason for this problem. Extensive advertising of stylish packaging and the presentation of this type of products in the form of fancy products can have a significant impact on increasing their level of public acceptance so; adverse organoleptic effects are reduced by careful selection of essential oils according to the type of food.

4. Conclusion

The results of this study displayed that the aqueous extract of *Alcea rosea* can be used as a potential source for pragmatic compounds to improve the retention of vitamin C and increase the content of phenolic compounds and antioxidant activity of tomato ketchup sauce. Also, it could therefore be that the rate of acceptance of sauce along with Hollyhock black extraction has been relatively satisfactory.

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