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European Journal of Experimental Biology, 2013, 3(5):307-312

ISSN: 2248 –9215
CODEN (USA): EJEBAU

The effects of adding extracts of Ziziphora (*Ziziphora tenuir*) as flavoring to chewing gums and the study of the release of caffeine from these extracts

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ABSTRACT

Caffeine is the most commonly used psychoactive material in beverages and foodstuffs in the world. Caffeine can be included as a medicine in the formulations of various foodstuffs such as chewing gums; however, its bitter taste poses a problem for manufacturers of food products. In this research, we have dealt with the release of caffeine from chewing gums during the chewing process and with the reduction of the bitteness of caffeine by using extracts of ziziphora. Moreover, we have carried out the sensory evaluation of ziziphora as a flavoring substance in chewing gums. Finally, we have compared the degrees of antioxidant activity of ziziphora using the two solvents methanol (80% v/v) and ethanol (80% v/v). The results obtained, indicated that by raising the caffeine content of chewing gums more of it is released from them, and that most of the caffeine in chewing gums is released during the first 5 to 15 min of their chewing. The results also showed that the use of ziziphora decreases the bitterness of caffeine, and that the antioxidant activity of ziziphora in the methanol solvent is greater than that in the ethanol solvent.

Key words: Antioxidant activity, Beverage, Caffeine, Foodstuff, Psychoactive, Ziziphora

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INTRODUCTION

Caffeine is an edible chemical substance found in various plants such as coffee, cocoa, cola and tea. It is an alkaloid of the methylxanthine family with characteristics similar to those of theophylline and theobromine. Pure caffeine is a bitter white powder formed from the combination of carbon, hydrogen, nitrogen, and oxygen. Caffeine is also used in formulations containing little amounts of sweeteners or no sugar. Strong sweeteners reduce the bitterness of caffeine. Compounds such as gluconates, sodium ascorbate, or other sodium salts combine with caffeine and lower high levels of caffeine bitterness, and thus raise customer satisfaction. Caffeine imparts a bitter flavor and this creates a problem for manufacturers wishing to expand the range of products that contain caffeine. The most potent materials in reducing the bitterness imparted by caffeine are 71% zinc lactate, 49% fat – free milk, and 31% sodium gluconate; while 32% chocolate and 17% coffee increase the bitter flavor of caffeine. Zin lactate is the most effective agent for lowering the bitter flavor of caffeine (23).

Ziziphora, with the scientic name of *Ziziphora tenuior*, is an annual, herbaceous, flocculent, slender, and erect plant with a height of 10 to 30 Cm. Its leaves are small, opposite, more or less lanceolate, and without petioles; the flowers are small and complete and red, pink or purple in color (2). Ziziphora is an aromatic plant and the most

important constituents of its essential oils include pulegone, limonene, and cineol (18). Its medicinal properties include a cure for gastrointestinal disorders such as diarrhea and gripes (8). Moreover, ziziphora has antioxidant (22) and antibacterial activities; it is also used as an intestinal antiseptic, an expectorant, and an anti – cold medicine (14). Studies have also been carried out concerning the stimulating effects of ziziphora essential oils on the immune system (3). Among the main active chemicals in this plant is a substance by the name of pulegone with well-known analgesic and anti - inflammatory effects (5). Pulegone is used to treat fever and dysmenorrhea and to preserve stomach tonus (8).There are some reports suggesting the analgesic effects of the plants of the mint family result from the presence of compounds such as carvacrol, flavonoids , and steroids (25). Ziziphora extracts lower blood pressure but do not substantially influence heartbeat.The essential oils present in ziziphora are volatile substances with curative effects as expectorant and carminative medicines , and as tonics for the stomach. The constituents of the essential oils in ziziphora have anti – tumor activities and reduce the growth of some malignant tumors by up to 36.6% and that of some cancerous glands by up to 47.5 % (1). These constituent essential oils are composed of mixtures of volatile chemical compounds and include terpenes, sesquiterpenes, their oxygenated derivatives, and other substances (20). Pulegone is the main constituent of the essential oils in some of the plants of the mint family such as ziziphora, it has antibacterial and antifungal properties (especially on the different strains of Salmonella), and is able to prevent the growth of Candida albicans and Salmonella typhimurium (its effect on Candida albicans is twice that of nystatin) (19).

MATERIALS AND METHODS

The chewing gum base, which was made in Turkey, was obtained from the Minoo Compay, and caffeine, sorbitol, xylitol, mannitol, glycerine, and lecithin from the Sigma Company. The essential oils of ziziphora were bought from the Exsir Gol- e Sorkh (Rose Elexir) Company in the province of Khorassan Razavi (the city of Mashhad). Caffeine was added at the three levels of 0.2, 0, 3, and 0, 4 gr to the base formulations of the chewing gums.

A. The method of chewing gum preparation

To prepare the chewing gums, the gum base was first placed in the laboratory mixer and kneaded for 6 min at 45 ℃ to obtain a sticky soft paste. The mixture of sweeteners, caffeine (at the three levels of 0.2, 0.3, and 0.4 gr), the emulsifier, and the ziziphora extracts were then gradually added. After mixing, kneading, and flattening the chewing gum paste, it was molded to take the shape of small circular pieces. After the chewing gum samples were prepared, they were packaged in plastic bags, randomly coded, and tested (8, 19, 20).

B. Measuring residual caffeine in chewing gum

A UV – vis (Milton Roy D, made in Korea) spectrophotometer was used at the wavelength of 273 nm to measure the remaining part of different concentrations of caffeine, which had been added to the chewing gum samples, after the chewing gums were chewed for different time periods (11).

C. Comparison of the antioxidant activities of chewing gums with ziziphora flavoring in the solvents ethanol and methanol

1. Preparation of the extracts

In this research , the solvents ethanol (80%V/V) and methanol (80% V/V) were used to compare the antioxidant activities of chewing gums to which ziziphora flavoring essential oils had been added. To the chewing gums prepared were added the three different levels of 0.25, 0.50, and 0.75 ml of the essential oils of ziziphora as flavoring. The chewing gums were then frozen and powdered. Ethanol and methanol (80%V/V) were employed to prepare extracts of ziziphora. 50 ml of each solvent were added to five grams of the powder and the resultant mixture was mixed for two hours at the ambient temperature using a magnetic mixer. The solid part was then removed by using ordinary filter paper and the extraction process was repeated as before using fresh solvent. The extracts obtained from these two steps were combined , concentrated at 40 ℃ by using a rotary evaporator (Model Laorata 4000 , made by the Heidolph Company) , and were finally dried at the ambient temperature.All materials used in this research were of a high degree of purity and they were obtained from the Merck Company. (14, 15)

2. The extent of capturing DPPH free radicals

To measure the extent of the capture of DDPH free radicals, 0.5 gr of the powders obtained from each of the solvents was dissolved in 50 ml of the respective solvent. .5 ml of the methanol solution of DDPH (0.5 mM) was added to 3.5 ml of the extract and the mixture was vigorously shaken. The test tubes were placed in the dark for 30 min. The degree of absorption at the wavelength of 517 nm was then read. It must be mentioned that in the control sample the extract was replaced with 3.5 ml of methanol. Finally, the percentage of the capture of the DPPH free radicals was calculated using the following formula:

The percentage of captured free radicals = $[\ A_c - \frac{A_s}{A_c}\ ] \times 100$

In the above relation: A_c and A_s are the control and the sample absorptions , respectively. (24)

3. Sensory evaluation

The sensory features of the chewing gum samples, including the reduction in the bitterness of caffeine by the extracts of ziziphora, the aroma, and the smell, and the stability of ziziphora essential oils in the chewing gums were investigated. Eleven trained evaluators were asked to express their views on the chewing gum samples. They gave scores to the degree of bitterness (1 = very bitter , 2= bitter , 3= average , 4= good , 5= very good) , to the aroma and smell (1= very little , 2= little , 3= average , 4= good , 5 = very good) , and to the stability of ziziphora essential oils (1 = very little , 2 = little , 3 = average , 4 = good , 5 = very good). In this method, the sensory tests mentioned were analyzed using the completely randomized statistical design, while taking the scores given by the evaluators into consideration. Duncan's multiple range test and the software Minitab 16 were employed to compare the means. (13)

RESULTS AND DISCUSSION

A. Residual caffeine in the chewing gums

Results show that at different times after the start of the chewing process, there are significant differences between the residual caffeine contents of the chewing gums to which different levels of caffeine are added. 5 min after the start of the chewing process, there are no statistically significant differences between the residual contents of caffeine in the chewing gums to which 0.3 gr of caffeiene is added and those to which 0.2 or 0.4 gr of caffeine is added. However, the differences between the chewing gums to which 0.2 or 0.4 gr of caffeine is added are statistically significant. (fig1) The reason for this difference is that with an increase in the dose of caffeiene added to the chewing gums the residual caffeine in these gums, decreases.The more caffeine is added to the chewing gums , the more caffeine is released. There is a statistically significant difference at 15 min after the start of the chewing process, too. With an increase in the length of time of chewing the gums, the residual caffeine contents of the gums decrease, and most of the added caffeine is released. After 15 min of chewing the gums, statistically significant differences are not observed between the treatments in which 0.2 or 0.3 gr of caffeine is added; nor are there statistically significant differences between chewing gums to which 0.4 gr of caffeine is added and they are chewed for 15 or 25 min. No statistically significant differences are observed between the treatments to which 0.2 or 0.3 gr of caffeine is added after 25 min of their chewing, but the differences between the other treatments are statistically significant. The reason for this is that with an increase in the level of caffeine added to the chewing gum (and with an increase in the length of time the gums are chewed), the residul contents of caffeine in the chewing gums decrease, and most of the added caffeine is released. Rosenhek et al. (1993) concluded that when the concentrations of saccrose and sodium bicarbonate in chewing gums increase, caffeine is released more rapidly from the first moments of chewing the gums. They also found that the hardness of chewing gums decreases with an increase in the length of time they are chewed; hence, more of the constituents of the gums are released after 5 min of chewing (16). Ochoa et al. (2008) showed that more caffeine is released from the chewing gums after 10 to 20 min of their chewing (6).

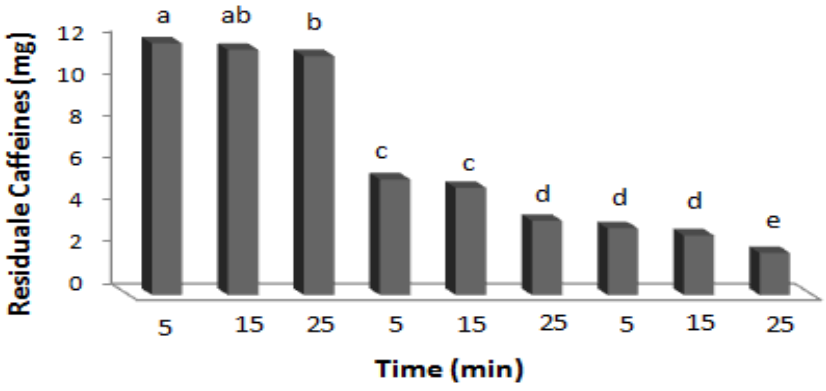

Fig1: Change of Residual caffeine in the chewing gums

B. Capturing DPPH free radicals

Results showed that with an increase in the levels of essential oils of used ziziphora as flavoring, the antioxidant activities of these substances increase. Moreover, the level of activities of the antioxidants present in extracts obtained by using the solvent methanol 80%, was higher than prepared extracts by employing the solvent 80%

ethanol.(Fig2) Kalithraka et al. studied the effects of different solvents in extracting various substances from grape seeds. They found that acetone and methanol extracted the largest amounts of procyanidins and catechins, respectively (24).These substances are among the most important antioxidants and are capable of reducing grape seeds; and, therefore, systems containing the solvents acetone and methanol have been able to perform better in the extraction of these substances. Systems containing acetone perform better in qualitative extraction, while those containing methanol do a little better in quantitative extraction. Optimization of the structure and the type of the solvent can play an important role in improving the extraction of antioxidant compounds (19,22).

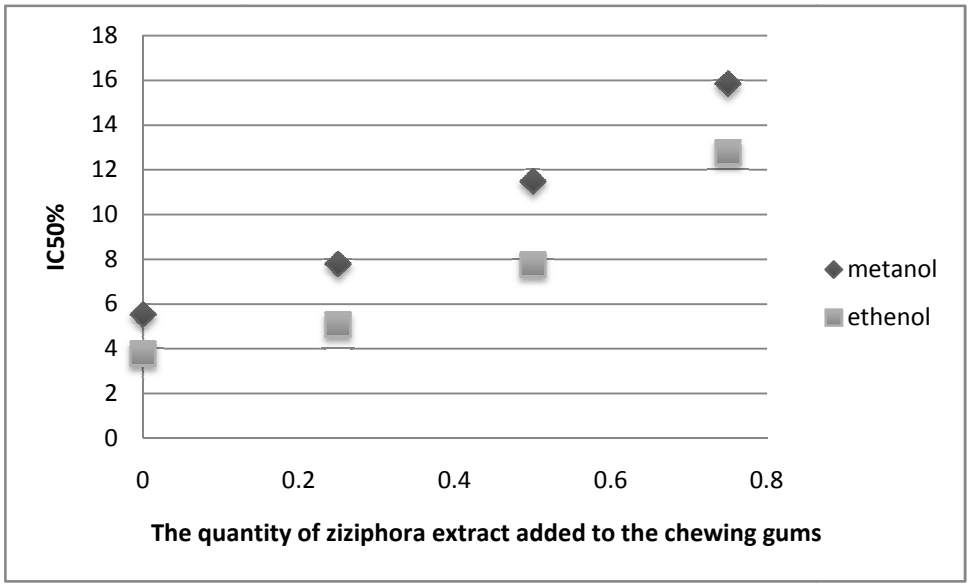

Fig2: Capturing DPPH free radicals

C. Sensory Evaluation

Results show , there are statistically significant differences between the treatments with respect to the reduction in the degree of bitterness of caffeine in chewing gums brought about by the use of ziziphora extracts. To chewing gum formulations each weighing 25 gr and containing 0.2 gr of caffeine was added 0.25, 0.5, or 0.75 mL extracts of ziziphora, respectively. Results obtained show that the degree of the bitterness of caffeine declines when more extracts of ziziphora are added to chewing gums. In fact, physico – chemical reactions can strengthen the flavor of chewing gums or even create new flavors in them. These events occur in a simple aqueous based solution: weak attracting bonds such as hydrogen or hydrophobic (lacking affinity for water) ones result in structural changes. The chemical compounds present on a food matrix will influence the perception of flavor (21). Lipids such as fats and fatty acids bring about a reduction in the bitterness (of caffeine) (17). When different compounds are mixed, the flavors of one or more of them change (23,25). For example, when sugar is added to coffee, the both sweetness of the sweeteners and the bitterness of caffeine decline.

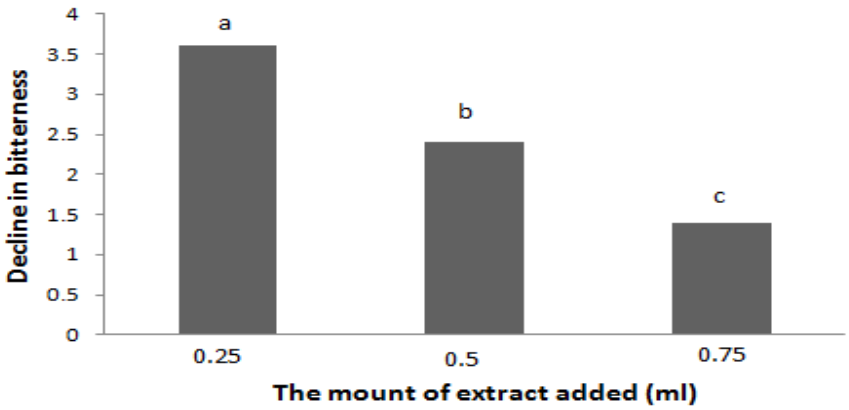

Fig 3: Reduction of the bitterness of caffeine by adding extracts of ziziphora

D. The aroma of the essential oils of ziziphora

Different amounts (0.07, 0.15, and 0.27 ml) of the flavoring essential oils in ziziphora were added to chewing gums. Figure 3 shows that there are statistically significant differencs between these treatments: the aroma is enhanced when more ziziphors essential oils are added to chewing gums. The reason for this is that when more ziziphora essential oils are added to chewing gums there will be more compounds imparting flavor and, hence, the smell of the chewing gums will be intensified. (7,13)

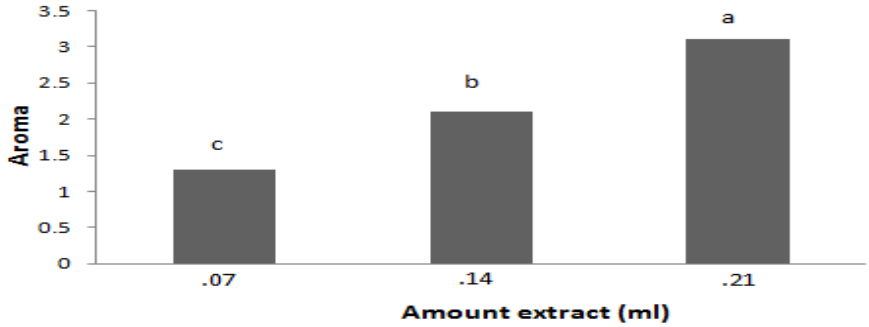

Fig 4: Study of the aroma of ziziphora essential oils against their concentrations

E. The stability of the essential oils of zizphora during 30 min of chewing gums

Result of the stability of the essential oils of ziziphora during 30 min of chewing gum indicates that there are statistically significant differences among the treatments with respect to the stability of the essential oils present in zizphora.(fig5) Results obtained by the panelists reveal that the stability of ziziphora essential oils increases when more of them are added to chewing gums.

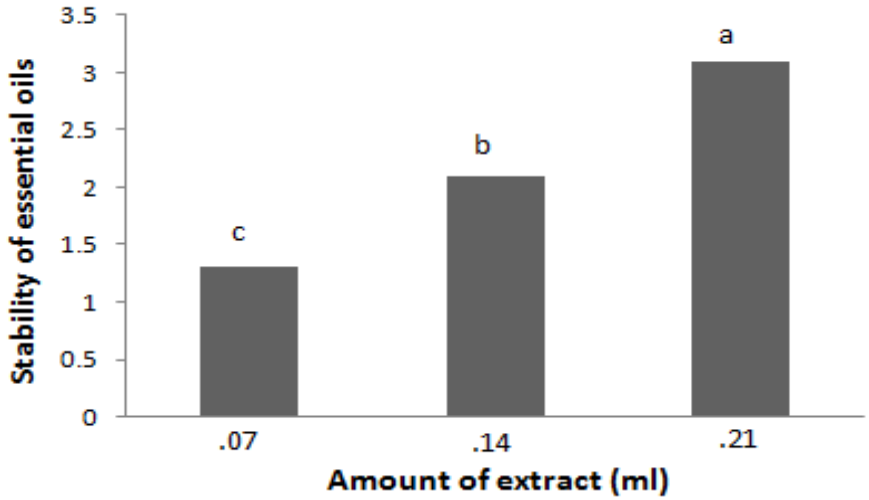

Fig 5: stability of essential oils of ziziphora

CONCLUSION

A. Since caffeine has medicinal properties, it can be used as a medicine in the formulations of chewing gums. The higher concentration of caffeine in chewing gums is, the greater the bitterness of the chewing gums will be (and the more of the caffeine will be released as compared with lower concentrations of caffeine in chewing gums).

B. More caffeine is released from the chewing gums after five to fifteen minutes of chewing them.

C. As caffeine increases the bitterness of chewing gums, the use of ziziphora extracts will lower the degree of bitterness; and when more of these extracts are used, this bitterness will decline further.

D. It was shown that using greater amounts of the natural essential oils of ziziphors as flavoring in chewing gums would increase the aroma and stability of the flavoring.

E. When methanol is used as the solvent, the antioxidant ctivities are greater than when ethanol is employed as the solvent. The extent of the antioxidant activities increases when more of the essential oils are used in chewing gums.

Table 1: Chewing gum formulations

Raw materials	Percentage by weight
Gum base	25 – 30
Sweetener	54 – 68
Lecithin	0.5
Glycerine	0.5
Flavoring	1

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