Original Article

Design, formulation and evaluation of green tea chewing gum

Abolfazl Aslani, Alireza Ghannadi[1], Zeinab Khalafi

Departments of Pharmaceutics, School of Pharmacy and Novel Drug Delivery Systems Research Center, [1]Pharmacognosy, School of Pharmacy and Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: The main purpose of this study is to design, formulate and evaluate the green tea gums with a suitable taste and quality in order to produce an anti-oxidant chewing gum.

Materials and Methods: Fresh green tea leaves obtained from Northern Iran for extraction. Maceration is the extraction method that is used in this study. The contents of caffeine, catechin and flavonoids of the hydro alcoholic extract were measured. Various formulations of the 120 mg green tea extract chewing gums with different sweeteners, flavoring agents and various gum bases were prepared afterward release pattern, content uniformity, organoleptic results and other properties were characterized.

Results: The contents of caffeine, catechin and flavonoid of the hydro alcoholic extraction were 207.32 mg/g, 130.00 mg/g and 200.82 mg/g, respectively. Release pattern of green tea chewing gum with different gum base ratios and various sweeteners in phosphate buffer were prepared. A total of 60 persons who were 20-30 years of age, participated in our panel test for organoleptic properties such as taste, stiffness, stickiness, etc., Acceptable gum was the one with the same ratio of the used rubber bases. Cinnamon selected as the preferred taste by volunteers. Combination of aspartame, sugar and maltitol has appropriate taste. The effect of various sweetener on release pattern was negligible, on the other hand rubber bases ratio variation, changed the release pattern obviously.

Conclusion: The green tea chewing gum with sugar, maltitol and aspartame sweeteners and cinnamon flavor, using the same rubber bases ratio may be a desirable antioxidant product.

Key Words: Caffeine, catechin, flavonoid, green tea chewing gum

Address for correspondence:
Dr. Zeinab Khalafi, Novel Drug Delivery Systems Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: Z_Khalafi_2000@yahoo.com
Received: 03.06.2013, **Accepted:** 08.09.2013

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.135159

INTRODUCTION

Several studies have been reported the effect of green tea consumption on health enhancement and prevention of cancer. Scientists' investigations on medium culture and animal studies confirm these results.[1] The beneficial effects of green tea leaves including prevention of cardiovascular diseases and

How to cite this article: Aslani A, Ghannadi A, Khalafi Z. Design, formulation and evaluation of green tea chewing gum. Adv Biomed Res 2014;3:142.

cancer are originated from tea polyphenol derivatives, including catechin with anti-oxidant effects. These compounds have free radical-scavenging activities. Free radical-mediated damage to DNA has been found in different cancer tissues.[2] Effective ingredients of green tea are useful to inhibit the carcinogenic stimuli, caused by ultraviolet (UV) radiations and carcinogenic chemicals. These ingredients have the protection role against lung, skin, oral cavity, esophagus, stomach, liver, pancreas, bladder, small intestine, colon, prostate and breast cancers.[1,3] Furthermore, prevention of incidence of diabetes mellitus type II, cardiovascular disease, stroke and heart failure, hypertension, hyperlipidemia, stress reduction and providing cavity hygiene by green tea ingredients are reported.[4,5]

Chemicals in green tea consist of polyphenols, caffeine and etc., Moreover, green tea leaves contain flavonoids, which are anti-oxidant. These flavonoids are effective in reducing inflammation, having antibacterial effect and preventing dental caries. Green tea consumption is diuretic, due to its caffeine content. In addition, because of theophylline contents, they can have therapeutic effects on respiratory diseases such as asthma.[4] Polyphenols exhibit *in vitro* antibacterial activity against periodontal pathogens; furthermore, they increase the anti-oxidant ability of oral fluids and prevent from periodontal diseases, moreover the polyphenol compounds decrease inflammation by creating an impenetrable layer with protein or polysaccharide.[6]

The anti-oxidant effects of catechin in green tea are more than the one of vitamin C and E, tocopherol and carotene.[4] Constituent materials in green tea causes tranquility by stimulating the gamma-aminobutyric acid (GABA) receptors in human central nervous system (CNS).[7] The active ingredients in green tea are also effective in weight loss that prevents diabetes mellitus and cardiovascular diseases in the long-term.[8]

Green tea is found in edible forms including raw form, 100, 250 and 500 mg tablets, 125, 675 and 850 mg chewable tablets, 100, 150, 175, 315, 333, 383 and 500 mg capsules, 600 mg caplets and in edible forms including topical lotions, creams, ointments and topical gels. Among these forms, the chewing gum form is missed.

Chewing gum form has predictable effects and lower side-effects, due to controlled drug release.[9] It increases sustained alertness and cognition while reduces chronic stress.[10] In comparison with tablets, chewing gum has fewer undesirable effects on CNS system performance and consciousness.[11] The time required for effecting the drug is faster in the case of chewing gum and the level of medicine in blood is hold steady for a long time in comparison with tablets.[12] This form of the drug is more acceptable by consumers, furthermore applying flavoring agents can increase acceptance.[12] Chewing gum form can better maintain the active ingredients, oxidable excipients and water absorber materials with an appropriate coating.[12] It also increases alertness, decreases tiredness and sleepiness.[13] Chewing gum form can be helpful in cleaning teeth surface while chewing and prevents forming teeth plaques[14] and helps dental cavity hygiene.[11,15,16]

Achievement of nicotine chewing gum in 1982,[17] introduced chewing gums as a new drug delivery system. For the first time in 1998, monographs of medicated chewing gums, accepted by European Pharmacopoeia as a pharmaceutical dosage form.[18] Nowadays these chewing gums are produced with a high standard as well as tablets. Today they can be formulated with various drug release profiles, while they are acceptable amongst different patients and clinicians.

Due to the importance of green tea and the presence of standard amounts of anti-oxidants of catechin, caffeine and flavonoid and the cancer prevention effects of these ingredients and the direct effect of green tea on preventing lots of diseases such as cardiovascular, weight gain, diabetes treatment and etc., the absence of green tea chewing gum among different types of green tea in the market is feeling. The main purpose of this study is to design, formulate and prepare the green tea chewing gum containing 10% of thickened green tea extraction, which in addition of being medicine, can be used as a natural anti-oxidant against disease and is acceptable by everyone including children.

MATERIALS AND METHODS

Materials

Gum bases such as elvasti, fruit C, 487 and stick were obtained from Gilan Ghoot Company, (Rasht, Iran). Flavoring agents of eucalyptus, cinnamon, peppermint and banana were from Goltash Company, (Isfahan, Iran) and flavoring agent of cherry from Farabi Pharmaceutical Company (Isfahan, Iran). Dried green tea leaves, which were the product of April 2011, received from Noor Jafari Company (Lahijan, Iran).

Materials such as maltitol, xylitol, aspartame, glycerol, aluminum chloride, potassium acetate, potassium dihydrogen phosphate, sodium hydroxide, glacial acetic acid, magnesium oxide, methanol, chloroform and perchloric acid prepared from Merck

Company (Germany). Dimethylamino cinnamaldehyde and Quercetin from Sigma-Aldrich Company (America) were prepared.

Methods

Green tea extract

Hydro-alcoholic extract of green tea obtained through maceration with 70% ethanol for 5 days. Alcohol of the extraction was removed on a rotary evaporator at 50°C. The residue was heated in a water bath at 40°C for further thickening. Quantification of caffeine, catechin and flavonoid contents in the extracted samples were then performed.[19]

Quantification of caffeine content in green tea extract

The caffeine content of green tea extracts was determined using spectrophotometric method. 3 g of magnesium oxide was added to 1 g of each prepared samples; 15 ml of boiling water were added to the mixture and allowed to stand in stirred boiling manner for 10 min. This mixture was then filtered using Whatman No 4 to obtain a clarified extract. Filtrate diluted with boiling water to a volume of 100 ml. 10 ml of buffered bisulphate solution (pH 6.0), which was diluted to 50 ml with water, was added to a 20 ml of an aliquot of the clarified extract and labeled solution "A". Another 20 ml of the clarified extract was placed in a separatory funnel and further extracted with 15 ml of chloroform. The chloroform was discarded and the residual contents of the separatory funnel were transferred to a beaker. The residual chloroform was removed by placing the beaker with its contents in a microwave oven set at 100°C for 5 min. The contents of the beaker were transferred to a calibrated flask and diluted with water to the 50 ml mark. This was labeled solution "B".

The absorbencies of solutions A and B were measured using a UV-visible (UV-VIS) spectrophotometer (Shimadzu, UVmini-1240) against distilled water at specific wavelengths. Caffeine content of a sample can be calculated using:

$$C_{Caffeine} = 10 \times \frac{E_a - E_b}{0.41.W.V}$$

Where C, W and V are caffeine content, weight of product sample (g) and volume of clarified extract (ml), respectively. E_a and E_b are absorbances for solutions A and B, respectively and can be calculated from: [20]

$$E_a \text{ and } E_b = E_{273} - \frac{(E_{250} + E_{296})}{2}$$

Quantification of catechin content in green tea extract

In order to prepare the samples for catechin analysis, 100 mg of green tea powder was refluxed at 70°C with 25 ml of 70% methanol for 45 min. The reflux was allowed to cool. It was then centrifuged and 1 ml of the particle-free supernatant was diluted with 70% methanol to the volume of 10 ml. In order to prepare the reagent, 26 mg of dimethylamino cinnamaldehyde was added to 25 ml of a mixture of 20 ml methanol, 2.5 ml perchloric acid and 2.5 ml water. Finally, 0.2 ml of the prepared sample was mixed with 0.5 ml of the prepared reagent and diluted with methanol to the volume of 5 ml. The UV absorption of the final prepared solution after 1.5 h was measured at 637 nm against 0.5 ml reagent, which was diluted with methanol to the volume of 5 ml as the blank. The quantity of catechin was calculated using an extinction coefficient of E (1%, 1 cm) = 141.[21]

Quantification of flavonoid content in green tea extract

The aluminum chloride colorimetric method was used.[22] Quercetin as a flavonoid was employed to make the calibration curve. First of all 10 mg of quercetin was dissolved in 80% ethanol and then diluted to the volume of 10 ml. After that the solutions of 2.5, 5, 10, 15, 20 and 25 µg/ml were prepared. 0.5 ml of the diluted standard solutions were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 molar potassium acetate and 2.8 ml of water. After incubation at room temperature for ½ h, the absorbance of the mixture was measured at 415 nm with the UV-VIS spectrophotometer. To prepare the blank solution, all substances except quercetin were used and 10% aluminum chloride was replaced. 10 mg of green tea extract was dissolved in 80% ethanol and then diluted to the volume of 10 ml. Finally, 0.5 ml of that was reacted with 10% aluminum and similar to the standard sample other steps were repeated. The experiment was repeated 3 times and the average of obtained absorbencies was calculated.

Preparation of formulations

First of all, gum bases were carefully weighed and heated up to 70°C in a water bath. Liquid glucose, glycerin and green tea extract was weighed exactly,

Table 1: Primary formulations of green tea chewing gum

Ingredients	Formulations				
	F_1	F_2	F_3	F_4	F_5
Gum bases					
Elvasti	80	100	80	80	90
487	100	100	80	100	90
Stick	80	80	100	100	90
Fruit C	100	80	100	80	90
Sugar	600	600	600	600	600
Green tea (powder)	120	120	120	120	120
Flavoring Agent	40	40	40	40	40
Glycerol	45	45	45	45	45
Liquid glucose	45	45	45	45	45

Table 2: Secondary formulations of green tea chewing gum

Ingredients	Formulations																	
	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	F_{17}	F_{18}	F_{19}	F_{20}	F_{21}	F_{22}	F_{23}
Gum bases																		
Elvasti	80	100	80	80	90	90	90	90	90	90	90	90	90	90	90	90	80	100
487	100	100	80	100	90	90	90	90	90	90	90	90	90	90	90	90	80	100
Stick	80	80	100	100	90	90	90	90	90	90	90	90	90	90	90	90	100	80
Fruit C	100	80	100	80	90	90	90	90	90	90	90	90	90	90	90	90	100	80
Sugar	600	600	600	600	600	600	500	300	400	500	400	400	400	-	300	300	400	400
Maltitol	-	-	-	-	-	-	-	-	-	-	200	200	-	400	300	200	200	200
Xylitol	-	-	-	-	-	-	-	300	200	100	-	-	200	200	-	100	-	-
Aspartame	-	-	-	-	-	4	4	4	4	4	4	4	4	4	4	4	4	4
Green tea (semisolid)	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120	120
Flavoring agent	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Glycerol	45	45	45	45	45	45	45	45	45	45	45	20	20	20	20	20	20	20
Liquid glucose	45	45	45	45	45	45	45	45	45	45	45	70	70	70	70	70	70	70

after that other powders were levigated, added to the gum bases and mixed well. Finally, flavoring agents were added to the prepared mixture at 40°C and left at room temperature to be cooled. In order to attain the green tea chewing gum with desired properties, the formulations listed in Tables 1 and 2 were prepared.

Among the prepared formulations F_{17} showed better properties, thus for further investigations on the drug release from gum bases, F_{22} and F_{23} were prepared.

Weight uniformity test

Single dose chewing gums were analyzed for weight uniformity. 20 samples weighed randomly and they did not differ more than 5% from their average.

Content uniformity test

In order to evaluate the green tea content uniformity of each sample, the content of caffeine in samples selected as the indicator. Firstly, 10 mg of pure caffeine dissolved in 100 ml chloroform then solutions of 5, 10, 15, 20, 25 and 30 μg/ml prepared. The wavelength of maximum absorbance (λ_{max}) for each standard solution was measured and then standard curve of caffeine standard in chloroform plotted.

For each prepared chewing gum samples, separately 10 specimens of equal weight dissolved in 100 ml of chloroform, centrifuged and absorption measurements were taken at a wavelength of the maximum absorbance of caffeine in chloroform using UV-VIS spectrophotometer.

Drug release test

The caffeine content selected as the indicator, in order to evaluate release of the green tea present in each chewing gum formulations. To gain the standard curve, 10 mg of pure caffeine dissolved in 100 ml of phosphate buffer, pH 6.8 and solutions of 5, 10, 15, 20, 25 and 30 μg/ml prepared. The wavelength of maximum absorbance for each standard solution was measured, after that standard curve of caffeine standard in phosphate buffer, pH 6.8 plotted.

A mastication device, which simulated the chewing gum masticating of human, was applied for drug release investigation. The device consisted of a masticating chamber, capable of holding the gum and the release medium (50 ml of phosphate buffer, pH 6.8) and a piston which stroked the gum (60 strokes per min). Water at 37 ± 0.5°C circulated in the jacket around the masticating chamber to avoid temperature changes. Sampling of mediums was performed at 5, 10, 15, 30, 45 and 60 min of mastication and absorption measurements were taken at a wavelength of the maximum absorbance of caffeine in phosphate buffer, pH 6.8 using UV-VIS spectrophotometer. In order to clarify the release mechanism and use the release results associated with each other, the mean dissolution time (MDT) was calculated by the following expression:[23]

$$MDT = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j}$$

Where j is the sample number, n is the number of dissolution sample times, is the time at the midpoint between t_j and t_{j-1} which can be calculated with the expression $(t_j + t_{j-1})/2$ and ΔM_j is the additional amount of drug dissolved between t_j and t_{j-1}. The MDT is a measure of the rate of the dissolution process; the higher the MDT, the slower the release rate.

Mechanical properties of chewing gums

Tensile investigations were performed on the last prepared formulations F_{21}, F_{22} and F_{23} which differed in the contents of gum bases, using a universal testing

machine (STM, Santam, Iran). Rectangular shaped tensile samples with a cross-section of 15 × 4 mm^2 and a gauge length of 50 mm, were fixed on gauges and were stretched under a constant strain rate of 50 mm/min at room temperature until breaking. The stress and strain were calculated based on elongation, applied force, initial sample length and cross section.

Evaluation of organoleptic characteristics of green tea chewing gums

Organoleptic properties such as chewing gum volume, softness/hardness, no stickiness to teeth and taste of different formulated green tea chewing gum tested with various volunteers. 10 trained volunteers tested the volume, softness/hardness, no stickiness to teeth and taste of green tea chewing gums under the supervision of the corresponding author. In order to be comparable, the defined features were assigned numbers of 1-5 using Likert scale.

Evaluation of the green tea chewing gums taste

A taste panel of 20 trained volunteers tested the organoleptic characteristics selected green tea chewing gum formulations, in the next step under the supervision of the corresponding author. In order to select the most desirable flavor by volunteers, a further taste panel performed. In this test, 30 trained volunteers compared the two top flavor of the former step.

RESULTS

Green tea extracts analyzing

About 230 ml of hydro-alcoholic extract of green tea obtained through maceration for 5 days from 100 g of green tea powder dissolved in 800 ml of 70% ethanol. Rotary and water bath were used to further concentration of the hydro-alcoholic extract and then kept refrigerated. A total of three samples of the extract of green tea were analyzed for caffeine, catechin and flavonoid contents, then, the average was calculated. Caffeine content and catechin content obtained 207.32 ± 1.5 mg/g and 130.00 ± 2.3 mg/g, respectively. Extract absorbance at 415 nm was 0.101 in which the flavonoid content calculated 200.82 ± 4.8 mg/g according to the standard curve of absorbance versus quercetin concentration, with the curve equation of y = 0.0061x – 0.0215 (R^2 = 0.9984).

Green tea chewing gum analysis

Weight uniformity investigation was performed on 20 randomly selected samples and the average weight calculated. The weight of F_{17} to F_{23} samples were in the range of 1.19-1.23 g. According to United States Pharmacopeia (USP) standard less than 5% should differ from the average and the results were entirely consistent with this standard.

Drug release analysis was performed separately on three specimens of each F_{17}, F_{18}, F_{19}, F_{20} and F_{21} formulations. The standard curve of absorbance versus caffeine in phosphate buffer, pH 6.8 concentration, caused the curve with the equation of y = 0.0672x – 0.0415 (R^2 = 0.9983) which was used to evaluate the caffeine content of green tea chewing gum in the release medium of phosphate buffer, pH 6.8. The averages of released caffeine during masticating versus time of sampling, with the consideration of 273.2 nm as λ_{max}, are illustrated in Figures 1 and 2 for samples with different sweeteners in formulations and different gum bases, respectively. The MDT values for F_{17}, F_{18}, F_{19}, F_{20}, F_{21}, F_{22} and F_{23} samples were calculated 5.12, 4.98, 4.78, 4.82, 4.85, 3.80 and 5.29, respectively.

A total of 10 separately specimens of each F_{17} to F_{23} samples were analyzed for caffeine content uniformity,

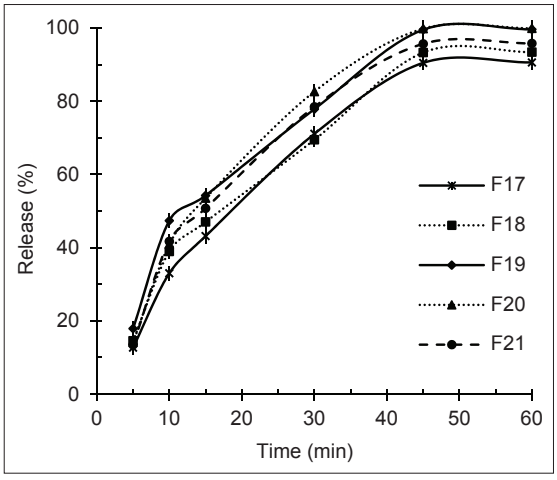

Figure 1: *In vitro* release of caffeine chewing gum formulations in pH 6.8 phosphate buffer at 37°C with various sweeteners

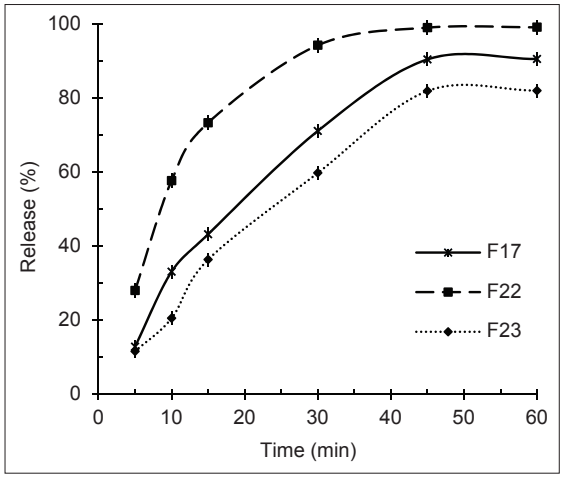

Figure 2: *In vitro* release of caffeine chewing gum formulations in pH 6.8 phosphate buffer at 37°C with various gum bases

using the standard curve of absorbance versus caffeine in chloroform concentration with the consideration of 272.0 nm as λ_{max}, caused the curve with the equation of y = 0.0648x – 0.0260 (R^2 = 0.9997). The averages of caffeine content in prepared formulations of F_{17}, F_{18}, F_{19}, F_{20}, F_{21}, F_{22} and F_{23} sample$_s$, were 130.5 ± 4.9, 129.3 ± 7.2, 131.8 ± 9.6, 130.9 ± 5.3, 132.2 ± 6.6, 134.1 ± 7.5 and 129.5 ± 9.4 μg/ml, respectively.

The averages of scores allocated by volunteers for each organoleptic property of samples are listed in Table 3. The summations of volunteers allocated scores of two taste panels are listed in Tables 4 and 5.

Tensile behavior of F_{21}, F_{22} and F_{23} samples, which consist of various gum bases, are illustrated in Figure 3.

DISCUSSION

The caffeine concentration in the green tea extract used in this study was 207.32 ± 1.5 mg/g. As shown by Mulder *et al.*,[24] the caffeine content of green tea and black tea was 57 and 55 mg/g, respectively. In addition, Vuong *et al.*[25] reported the value of 95 mg/g for caffeine content in green tea leaves. The difference in the reported caffeine contents and the measured caffeine content in this study may mainly relate to apply green tea extract in this work, in comparison with green tea leaves in other studies. Furthermore,

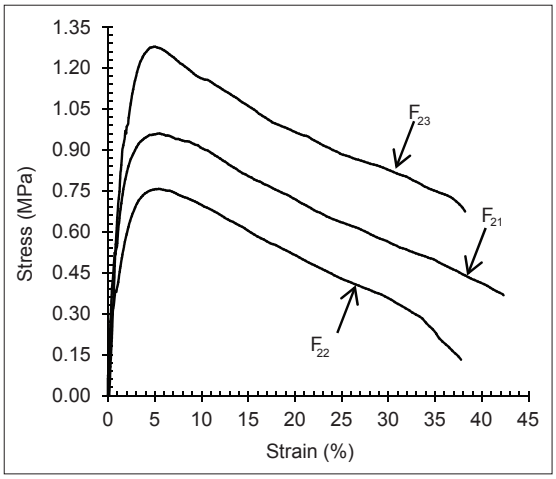

Figure 3: Tensile behavior of F_{21}, F_{22}, and F_{23} formulations with various gum bases

Table 3: The averages of scores allocated by volunteers for organoleptic properties of green tea chewing gum formulations by 10 volunteers

Formulations	Chewing gum volume[1]	Softness and Hardness[2]	Not stickiness[3]	Taste[4]
F_1	2	5	4.3	1.2
F_2	2	5	4	1
F_3	2	5	4.3	1
F_4	2	5	4.1	1.1
F_5	2	5	4	1.2
F_6	3	2	4	1.8
F_7	3	4	4.4	1.5
F_8	3	2	3	1.7
F_9	3	2	4.7	1.6
F_{10}	3	3	4.7	1.8
F_{11}	3	3	4.9	3.8
F_{12}	3	3	4.6	2.8
F_{13}	3	3	4.5	2.3
F_{14}	3	3	4.7	2.6
F_{15}	3	3	4.8	3.5
F_{16}	3	3	4.8	4.9
F_{17}	3	3	5	5
F_{18}	3	3	4.9	4
F_{19}	3	3	4.9	3.8
F_{20}	3	3	4.9	4.7
F_{21}	3	3	4.9	4.5
F_{22}	3	3	3.2	4.9
F_{23}	3	3	4.9	5

[1]The bulk volume of gum was evaluated as Huge=5, much=4, right=3, little2, very little=1, [2]The Softness/Hardness was evaluated as very hard= 5, hard= 4, suitable=3, soft=2, very soft=1, [3]The stickiness to teeth was evaluated as never sticks=5, rarely sticks=4, sometimes sticks=3, mostly sticks=2, always sticks=1, [4]The Taste was evaluated as excellent=5, good=4, fair= 3, poor= 2, very poor= 1

Table 4: The summations of scores allocated by 20 volunteers for each taste of best green tea chewing gum formulations

Flavoring agents	Formulations				
	F_{17}	F_{18}	F_{19}	F_{20}	F_{21}
Cherry					
Sum	62	44	65	60	79
Median	3	2	3	3	4
Banana					
Sum	74	58	64	81	53
Median	4	3	3	4	2.5
Eucalyptus					
Sum	52	48	67	58	58
Median	2.5	2.5	3.5	3	3
Peppermint					
Sum	87	78	85	83	82
Median	4	4	4	4	4
Cinnamon					
Sum	91	86	92	87	90
Median	4.5	4	4.5	4	4.5

Table 5: The summations of scores allocated by 30 volunteers for top tastes of best green tea chewing gum formulations

Flavoring agents	Formulations				
	F_{17}	F_{18}	F_{19}	F_{20}	F_{21}
Peppermint					
Sum	135	114	125	131	132
Median	4.5	4	4	4	4.5
Cinnamon					
Sum	140	122	134	133	139
Median	4.5	4	4.5	4.5	4.5

various measuring methods and different source of green tea can be impressive.

The green tea extract used in this study consisted of 130.00 ± 2.3 mg/g catechin. Ye *et al*.[26] reported the value of 133.25 ± 1.19 mg/g for the total catechin in green tea leaves. As shown by Chandra *et al*.,[27] the catechin content of green tea leaves was 137.16 ± 5.79 mg/g. Catechin content of green tea leaves in another research by Henning *et al*.[28] was 204.7 mg/g. Pelillo *et al*.[29] reported various values of 93, 697 and 757 mg/g for 3 different commercial green tea extracts.

The flavonoid concentration in the green tea extract used in this study was 200.82 ± 4.8 mg/g. Total polyphenol of green tea as Kim *et al*.[30] reported was in the range of 475.6-811.1 mg/g; While Lee *et al*.[31] expressed the range of 244.7-368.5 mg/g. Various values may attribute to the different source of green tea and quantification method. Furthermore, in some studies fresh green tea leaves were employed while in this work extract of dried green tea powder was applied.

The extract of dried green tea powder with the above mentioned characteristics used to prepare various formulations of green tea chewing gum. Among lots of ingredients in green tea, caffeine content selected as an indicator of green tea release.

In F_1, F_2, F_3, F_4 and F_5 samples, which prepared from green tea powder, the volume of chewing gums were relatively lower, since the powder type substances amount were more than gum bases. They scraped while chewing and were not like a real chewing gum. Changing the gum bases did not resolve the problem. The bitter taste of these chewing gums was obvious. Consequently green tea powder was replaced by green tea concentrated extract in formulations and samples of F_6, F_7, F_8, F_9 and F_{10} were prepared. Among others the F_{10} sample with the same amount of gum bases, had better organoleptic properties. However, still the bitter taste was sensible. Adding 4 mg of aspartame to the former resulted F_{11} sample and mostly covered the bitter taste, although aspartame is one of the most effective sweeteners, it could not completely omit the bitter taste and because of the limitation of using sweeteners, it was not possible to use extra sweeteners. In the F_{11} sample, which most of the bitter taste was vanished, the sugar content in F_{12} was lessened, but it was not affective. The attempt to replace half of sugar content by xylitol in F_{13} sample was not successful. In F_{14} and F_{15} samples more contents of sugar were replaced by xylitol but the bitter taste was not omitted, the only merit was decreasing the sugar and increasing xylitol with more benefits. In F_{16} sample maltitol was used in combination with sugar and aspartame and the bitter taste decreased. In F_{17} sample, the amount of liquid glucose increased and glycerol decreased. Not only the taste of this sample was desirable, but also the organoleptic properties of that were appropriate. In order to investigate the effect of various sweeteners, the samples of F_{18}, F_{19}, F_{20} and F_{21} were prepared. All of them had acceptable tastes, but the F_{17} sample had the most appropriate taste among the others.

As it is demonstrated in Figure 1, the drug release patterns of samples with various sweeteners (F_{17}, F_{18}, F_{19}, F_{20} and F_{21}) are similar and the MDT values which are about 4.9, confirm this. At 5, 10, 15, 30 and 45 min after masticating, the drug release was 15, 40, 50, 76 and 96%, respectively. The slight difference may relate to different sweetener contents with alcoholic structures (maltitol and xylitol) in chewing gum formulation. Samples of F_{17} and F_{18} consist of 200 mg, F_{20} and F_{21} consist of 300 mg and F_{19} consist of 600 mg of alcoholic sweeteners. The drug release of caffeine chewing gum at 10, 20 and 30 min reported by Aslani *et al*.[32] was 55, 78 and 89%, respectively. Another study by Tyrpin *et al*.[33] reported the drug release of caffeine coated chewing gum at 20 min of 88% and at 40 min of 97%. The variation in the measured values and the reported values may relate to different substances in green tea, which can affect the caffeine release. In another study by Aslani *et al*.[34] on nicotine chewing gum, although the employed gum bases were similar to the gum bases used in the current study, the drug release of majority of samples at 30 min were in the range of 79-83% of the affective substances. Kvist *et al*.[35] reported a complete release of nicotine from 2 mg nicotine chewing gum in about 45 min. Another study from Kvist *et al*.[36] on 20 mg dimenhydrinate chewing gum showed about 90% release of dimenhydrinate from the gum in about 45 min. The drug release from chewing gum is dependent on its water solubility, the more water-soluble substance is, the more release from chewing gums occurs and vice versa.[15]

Samples of F_{22} and F_{23} with various gum bases were prepared in order to compare the drug release pattern of these gum bases with the F_{17} sample. As it is demonstrated in Figure 2, the drug release pattern of F_{22} sample, which consists of softer gum bases (fruit C and stick), in the same time shows more caffeine release in comparison with F_{17} and F_{23}, which consist of lower content of those soft gum bases. The MDT value of F_{22} sample is less than F_{17} and F_{23} samples that means the release rate for this formulation is higher than others. The Figure 2 also illustrates that the F_{23} sample, which consists of harder gum bases (elvasti

and 487), in the same time exhibits less caffeine release in comparison with others and the MDT value of this formulation is higher, which intends slower release rate. Consequently, the samples with softer gum bases showed more release in the same times.

The drug release kinetics of the samples was calculated. The zero order release constant (K_0) for samples with various sweeteners of F_{17}, F_{18}, F_{19}, F_{20} and F_{21} were 1.554, 1.556, 1.615, 1.685 and 1.605 μg/(ml.min) with the *r*-squared values (R^2) of 0.908, 0.902, 0.871, 0.877 and 0.874, respectively. On the other hand, the zero order release constant for samples with various gum bases of F_{17}, F_{22} and F_{23} were 1.554, 1.477 and 1.449 μg/(ml.min) with the *r*-squared values of 0.908, 0.732 and 0.935, respectively.

The first order release constant (K_1) for samples with various sweeteners of F_{17}, F_{18}, F_{19}, F_{20} and F_{21} were 0.008, 0.009, 0.019, 0.024 and 0.011 min^{-1} with the (R^2) values of 0.962, 0.951, 0.917, 0.925 and 0.959, respectively. While, the values of 0.008, 0.017 and 0.006 min^{-1} were obtained for the samples with various gum bases of F_{17}, F_{22} and F_{23} with the *r*-squared values of 0.962, 0.967 and 0.961, respectively.

As all the *r*-squared values for the first order model were near one, the drug release kinetics of the gums is the first order.

The content uniformity results confirmed that the caffeine contents of various samples are near each other.

Organoleptic investigations showed that F_{16}, F_{17}, F_{18}, F_{19}, F_{20}, F_{21}, F_{22} and F_{23} samples have appropriate organoleptic properties among the others. The samples of F_{17}, F_{18}, F_{19}, F_{20}, F_{21}, F_{22} and F_{23} elected for taste evaluation. Cinnamon and peppermint were more preferable flavors among the other flavors. It is notable that eucalyptus taste affected the gum base and induced extra softening in chewing gum. Banana and cherry were not able to cover the bitter taste of green tea. Cinnamon has a sweet taste itself in comparison with other flavoring agents and the taste of chewing gum became more acceptable while using cinnamon. The use of aspartame is reported to cover the bitter taste of nicotine chewing gum as well as green tea, but with different flavoring agents of cherry and eucalyptus.[34]

As illustrated in Figure 3, the F_{21}, F_{22} and F_{23} samples demonstrated ductile behavior. As the constant strain rate was applied, the samples displayed linear elastic behavior, defined by a linear stress-strain relationship, in which deformations are completely recoverable upon removal of the load; that is, the specimens show elastic behavior at these elongations. As the tension increases, nonlinear behavior appears and deformations are plastic. It means that the plastically deformed specimens do not completely return to their original size and shapes when unloaded. More strain causes yielding followed by stress softening, in which the stress decreases with increasing strain. Elongating more tended the specimens to break. All three samples have similar Young modulus of around 0.7 MPa. The yield points of F_{21}, F_{22} and F_{23} samples occur at the stresses of 0.96, 0.76 and 1.28 MPa and strains of 5.46, 5.45 and 4.98%, respectively. The yield point for the F_{23} sample with more elvasti and 487 bases take place at higher stresses, in contrast, for the F_{22} sample with more fruit C and stick bases, lower yield point among the other samples happen. It is notable that the linear elastic portion of F_{23} sample is more than the one for others and F_{22} sample is lowest. According to these mechanical properties it can be predict that due to the same young modulus, all samples show similar stiffness while chewing. Furthermore, the F_{23} sample may sustain its chewable manner as it has higher yielding and behaves more elastic as it has larger elastic range. The F_{22} sample may lose its chewability due to lower yielding and behaves more plastic as it has lower elastic range. The F_{21} sample has a medial behavior.

CONCLUSION

The extracted green tea contained 207.32, 130.00 and 200.82 mg/g of caffeine, catechin and flavonoid, respectively, depicts that the Iranian green tea used in this study includes an appropriate range of effective ingredients. The caffeine release of chewing gums with softer gum bases were more than the ones with harder gum bases at the same times. It was also observed that samples with the same gum bases, but with various formulations had similar caffeine release pattern and the drug release kinetics followed the first order model. Tensile analysis of three samples with different gum bases showed that the samples broke after yield point under tension. The prepared chewing gums had various organoleptic properties. The sample with equal content of gum bases had appropriate organoleptic properties. A combination of sugar, aspartame and maltitol covered the bitter taste of the green tea. The selected tastes by volunteers were cinnamon and peppermint, respectively.

REFERENCES

1. Landau JM, Lambert JD, Yang CS. Green tea. Nutritional Oncology. 2nd ed. Burlington, Ma: Academic Press; 2006.
2. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging 2007;2:219-36.
3. Rosengren RJ. Utility of epigallocatechin gallate in the treatment and prevention of breast cancer: molecular mechanisms for tumor suppression. Handbook of Green Tea and Health Research, Eds: McKinley H and Jamieson M; 2009.

4. Sharangi AB. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. Food Res Int 2009;42:529-35.
5. Egashira N, Mishima K, Iwasaki K, Oishi R, Fujiwara M. Neuroprotective effect of theanine on cerebral ischemia. Handbook of Green Tea and Health Research, Eds: McKinley H and Jamieson M; 2009.
6. Aslani A, Ghannadi A, Najafi H. Design, formulation and evaluation of a mucoadhesive gel from *Quercus brantii* L. and *Coriandrum sativum* L. as periodontal drug delivery. Adv Biomed Res 2013;2:21.
7. Furuse M, Adachi N, Tomonaga S, Yamane H, Denbow DM. Central functions of green tea components. Handbook of Green Tea and Health Research, Eds: McKinley H and Jamieson M; 2009.
8. Yun CH, Kim GR, Seo MJ, Moon HS, Cho CS. Anti-obesity effects of (-)-epigallocathchin 3-gallate and its molecular mechanism. Handbook of Green Tea and Health Research, Eds: McKinley H and Jamieson M; 2009.
9. Golding JF. Motion sickness susceptibility. Auton Neurosci 2006;129:67-76.
10. Allen AP, Smith AP. A review of the evidence that chewing gum affects stress, alertness and cognition. J Behav Neurosci Res 2011;9:7-23.
11. Seibel K, Schaffler K, Reitmeir P, Golly I. A randomised, placebo-controlled study comparing two formulations of dimenhydrinate with respect to efficacy in motion sickness and sedation. Arzneimittelforschung 2002;52:529-36.
12. Reiner A, Seneci A. Pharmaceutical compositions based on chewing gum and a method for the preparation thereof. US Patent 1998; [5,711,961].
13. Johnson AJ, Miles C, Haddrell B, Harrison E, Osborne L, Wilson N, *et al*. The effect of chewing gum on physiological and self-rated measures of alertness and daytime sleepiness. Physiol Behav 2012;105:815-20.
14. Miskewitz RM. Chewing gum product with dental health benefits. US Patent 1997; [5,693,334].
15. Rassing MR. Chewing gum as a drug delivery system. Adv Drug Deliv Rev 1994;13:89-121.
16. Deshpande A, Jadad AR. The impact of polyol-containing chewing gums on dental caries: A systematic review of original randomized controlled trials and observational studies. J Am Dent Assoc 2008;139:1602-14.
17. Parrott AC, Winder G. Nicotine chewing gum (2 mg, 4 mg) and cigarette smoking: Comparative effects upon vigilance and heart rate. Psychopharmacology (Berl) 1989;97:257-61.
18. William PV, Millind T. A comprehensive review on: medicated chewing gum. Int J Res Pharm Biomed Sci 2012;3:894-907.
19. Ghannadi A, Hajhashemi V, Abrishami R. Effects of the Persian *Carum copticum* fruit extracts on morphine withdrawal syndrome in mice. Res Pharm Sci 2012;7:127-31.
20. Eteng MU, Eyong EU, Eka OU, Umoh IB, Ebong PE, Ettarh RR. Caffeine and theobromine levels in selected Nigerian beverages. Plant Foods Hum Nutr 1999;54:337-44.
21. Kivits G, Sman F, Tijburg L. Analysis of catechins from green and black tea in humans: a specific and sensitive colorimetric assay of total catechins in biological fluids. Int J Food Sci Nutr 1997;48:387-92.
22. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
23. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13:123-33.
24. Mulder TP, Rietveld AG, van Amelsvoort JM. Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. Am J Clin Nutr 2005;81:256S-60.
25. Vuong QV, Golding JB, Nguyen MH, Roach PD. Preparation of decaffeinated and high caffeine powders from green tea. Powder Technol 2013;233:169-75.
26. Ye JH, Liang YR, Jin J, Liang HL, Du YY, Lu JL, *et al*. Preparation of partially decaffeinated instant green tea. J Agric Food Chem 2007;55:3498-502.
27. Chandra S, De Mejia Gonzalez E. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (Ilex paraguariensis) and green (*Camellia sinensis*) teas. J Agric Food Chem 2004;52:3583-9.
28. Henning SM, Fajardo-Lira C, Lee HW, Youssefian AA, Go VL, Heber D. Catechin content of 18 teas and a green tea extract supplement correlates with the antioxidant capacity. Nutr Cancer 2003;45:226-35.
29. Pelillo M, Biguzzi B, Bendini A, Toschi TG, Vanzini M, Lercker G. Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins. Food Chem 2002;78:369-74.
30. Kim SY, Jeong SM, Jo SC, Lee SC. Application of far-infrared irradiation in the manufacturing process of green tea. J Agric Food Chem 2006;54:9943-7.
31. Lee SC, Kim SY, Jeong SM, Park JH. Effect of far-infrared irradiation on catechins and nitrite scavenging activity of green tea. J Agric Food Chem 2006;54:399-403.
32. Aslani A, Jalilian F. Design, formulation and evaluation of caffeine chewing gum. Adv Biomed Res 2013;2:72.
33. Tyrpin HT, Russell MP, Witkewitz DL, Johnson SS, Ream RL, Corriveau CL. Caffeine coated chewing gum product and process of making. US Patent 2002; [6,444,241].
34. Aslani A, Rafiei S. Design, formulation and evaluation of nicotine chewing gum. Adv Biomed Res 2012;1:57.
35. Kvist C, Andersson SB, Fors S, Wennergren B, Berglund J. Apparatus for studying *in vitro* drug release from medicated chewing gums. Int J Pharm 1999;189:57-65.
36. Kvist LC, Andersson SB, Berglund J, Wennergren B, Fors SM. Equipment for drug release testing of medicated chewing gums. J Pharm Biomed Anal 2000;22:405-11.

Source of Support: The authors appreciate Isfahan University of Medical Science Vice Chancellery For Research that supported us financially. **Conflict of Interest:** No.