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ORIGINAL PAPER

Development of a Natural Chewing Gum from Plant Based Polymer

Ibrahim Palabiyik[1] · Omer Said Toker[2] · Nevzat Konar[3] · Barış Öner[1] · Ahmet Sukru Demirci[1]

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Abstract In this study, Kenger gum obtained from Kenger plant (*Gundelia tournefortii*) was used in the production of biodegradable and edible chewing gum. Kenger gum was able to be softened by thermal process to improve its textural properties. 80% methanolic extract of gum showed 195.6 gallic acid equivalents (GAE) mg/100 g gum antioxidant activity and 17.9 mm inhibition zone for *Escherichia coli* O157:H7 as an antimicrobial activity. Softened Kenger gum was also characterized by texture properties, scanning electron microscope (SEM) images and chemical compositions. Hardness value of gum decreased from 864 to 238 g which was comparable to commercial chewing gums. Softened Kenger gum was observed to be a perfect substitute for a synthetic gum base in the production of a conventional chewing gum. Moreover, resilience value was remarkably found to be the best standard parameter to select chewing gums with desired textural properties.

Keywords Kenger gum · Antioxidant activity · Antimicrobial activity · Textural properties

✉ Ibrahim Palabiyik
ipalabiyik@yahoo.com

[1] Food Engineering Department, Agricultural Faculty, Namik Kemal University, 59030 Tekirdağ, Turkey

[2] Food Engineering Department, Chemical and Metallurgical Engineering Faculty, Yildiz Technical University, Istanbul, Turkey

[3] Department of Food Engineering, Faculty of Architecture and Engineering, Siirt University, 56100 Siirt, Turkey

Introduction

Chewing gum is a popular product around the world and chewed by people at all ages for mainly enjoyment. In recent years, it has been used for therapeutic purpose involving oral hygiene and smoke alternative [1]. Therefore, the production of chewing gum having positive effects on health has drawn attention as a current issue of the food industry. For this aim ingredients having negative effect on health and environment in the chewing gum formulation should be replaced by alternative substances. Considering the formulation of chewing gum, the main ingredient causing problem in this respect is gum base which plays an important role in the manufacturing of chewing gum with desired chewiness and textural characteristics [2, 3]. The efforts should be focused on replacement of chewing gum base which is composed of rubbers such as styrene butadiene, butyl or polyisobutylene.

According to the Euromonitor International Co. Ltd, the value of chewing gum sales was stated as 25 billion dollars. The statistics disclosed that average chewing gum consumption in a year was 160–180 numbers per person. This large consumption resulted in 250,000 tones waste which can be cleaned by spending a huge amount of money. Therefore, substitution of artificial gum base with natural and biodegradable one has great importance in many aspects. In the previous studies, several researchers investigated to utilize zein protein instead of gum base in the chewing gum formulation [4]. The findings highlighted that zein protein can be used as an alternative to gum base; however, further studies are required to produce chewing gum with desired sensorial properties. In the present study, Kenger plant latex was used as a chewing gum base.

Kenger gum is produced by solidification of milk flowing as a result of cutting the root of Kenger plant (*Gundelia tournefortii*) (Fig. 1). The plant is grown naturally

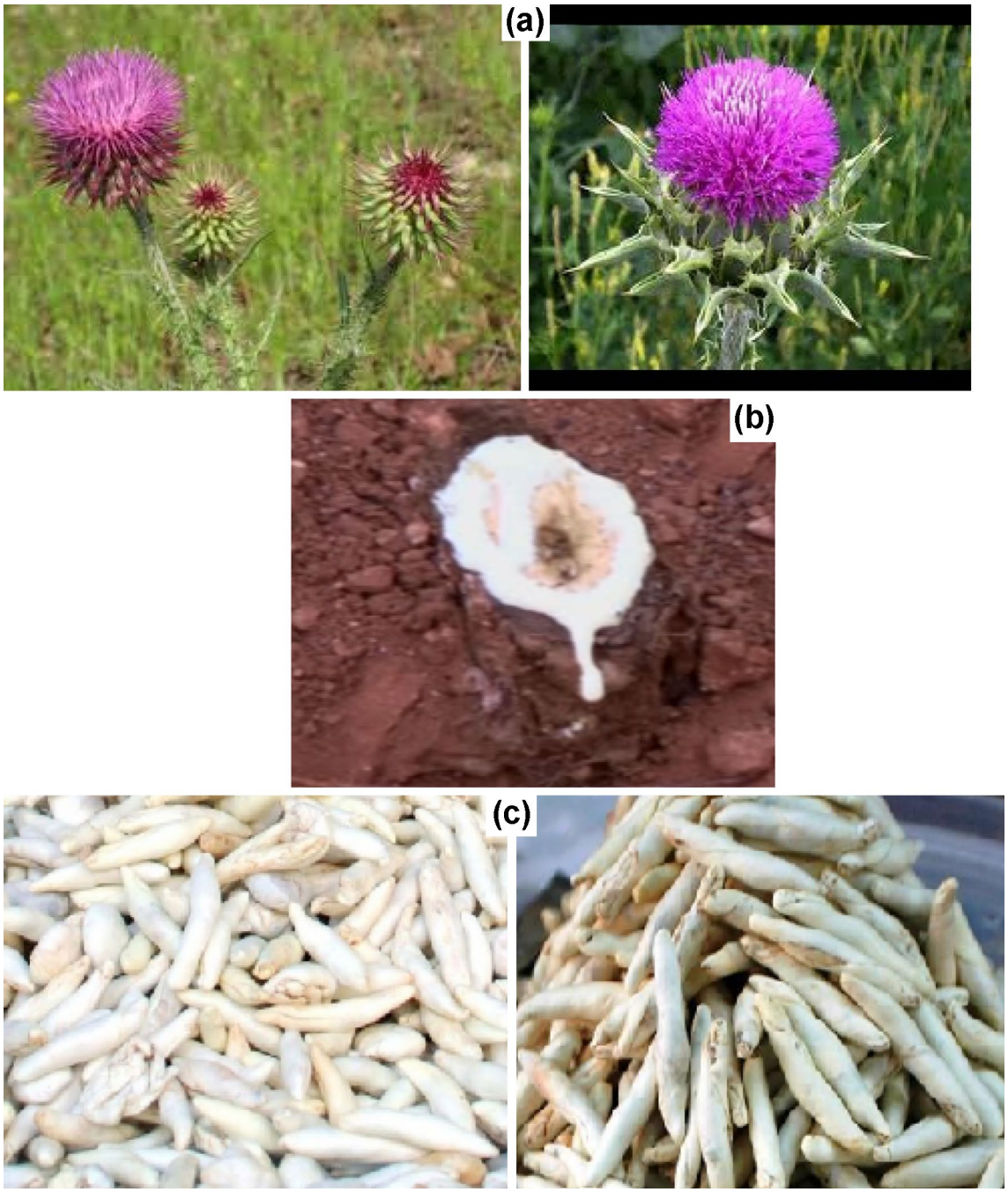

Fig. 1 Kenger plant (*G. tournefortii*) (**a**), its milk (**b**) and kenger chewing gum (**c**)

in Eastern Anatolia, Southeastern Anatolia and Mediterranean regions of the Turkey and traditionally believed to be remedy for various diseases such as toothache, gingiva pains, cramp, indigestion, migraine and embolism. Although Kenger gum provides many beneficial effects, its hard texture restricts consumption of it since it causes difficulty during chewing, which decreases preferability of the product. Therefore, in the present study, production of natural chewing gum by using Kenger gum as an alternative to artificial gum base was investigated.

The work is the first study related with softening of the Kenger gum and using it in the production of natural chewing gum instead of gum base, which has potential to direct future works.

Materials and Methods

Materials

Unprocessed and traditionally produced untreated Kenger gum was obtained from the local retailer, Malatya, Turkey. It was stored in water prior to analyses and until processing of the Kenger gum to the other products.

Some Physicochemical and Functional Properties of Untreated Kenger Chewing Gum

Determination of Trace Elements

Elemental analyses were conducted by inductively coupled plasma optical emission spectrometry ICP-OES (Spectro Blue, Germany). Quantitative analyses of samples were carried out according to the calibration technique. For this aim, standard calibration solutions were prepared as a result of diluting multi-element standard solution containing analyte elements. Five different concentrations of analytes were used to determine detection limit. All the measurements were performed using the full quantitative model analysis. The correlation coefficients calculated for each analytes were at least equal or higher than 0.995.

Dry Matter and Ash Contents

Dry matter content of Kenger gum was determined according to the method described by Ludorf and Meyer [5]. It was dried at 105 °C until the constant weight was obtained. Ash content was determined as a result of incinerating the sample at 550 °C for 4 h until grey colour was observed.

Crude Protein Content

The method described by Mattissek et al. [6] was performed to determine protein content of the Kenger gum. After putting 1 g sample to Kejldahl bottle, 2 g catalyst ($K_2SO_4 + Cu_2SO_4$ mixture) and 10 mL H_2SO_4 were added. The prepared mixture was burned at 420 °C until transparent light green color was observed. After cooling of the mixture to room temperature, 50 mL distilled water and NaOH (33%) were added. 35 mL H_2SO_4 (N/7) and three drops methyl red (0.1% in alcohol) were put into Erlenmeyer flask and distillation proceeded until 100 mL mixture was achieved in the Erlenmeyer. After titrating of the mixture with NaOH (N/7), crude protein content was determined.

Antimicrobial Activity

Microbial Strains

The extracts of Kenger gum was obtained by centrifugation of Kenger gum which were cut into small pieces for 30 min in the corresponding solvents. Extracts were individually tested against five pathogenic microorganisms: *Escherichia coli* O157:H7 ATCC 33150. *Listeria monocytogenes* ATCC 7644. *Salmonella enterica* subsp. *enterica* serovar Enteritis ATCC 13076. *Staphylococcus aureus* ATCC 2592 and *Vibrio parahaemolyticus* ATCC 17802. All the strains mentioned above were obtained as actively growing cultures from the American Type Culture Collection (ATCC). Stock cultures of all the strains were grown in Nutrient Broth (Merck) at 37 °C for 24 h and suspensions were adjusted to 0.5 McFarland standard turbidity (each bacterial suspension included about 10^7–10^8 cells).

Disc Diffusion Assay

The agar disc diffusion method was employed for the determination of antimicrobial activity of Kenger extracts [7]. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cfu per mL) was spread on Mueller Hinton Agar (MAH) medium. Filter paper discs (6 mm in diameter) were impregnated with 20 µL of the extracts or methanol (negative control) and placed on the inoculated plates. These plates were stored at 4 °C for 2 h to enable prediffusion of the extracts into the agar and then incubated at 37 °C for 24 h for the observation of bacterial growth. The diameters of the inhibition zones were measured in millimeters. All tests were performed in duplicate.

Determination of Total Phenolic Content

The total phenolic content was determined by using the Folin–Ciocalteu reagent and gallic acid as the standard as described previously with some modifications [8]. Briefly, 5 mL water, 1–3 mL sample and 0.5 mL Folin–Ciocalteu reagent were mixed and incubated for 5–8 min at room temperature. 1.5 mL sodium carbonate (20%. w/v) was added to obtain a final volume of 10 mL. The solution was mixed and incubated for 2 h and filtered (0.45 µm polytetrafluoroethylene filter, Whatman) prior to reading the absorbance at 750 nm in a spectrophotometer (Shimadzu UV–Vis Mini 1240). The total phenol content was quantified by comparing the absorbance of the samples with the absorbance of the gallic acid standard. A calibration curve with gallic acid was prepared in the 5–25 mg/L range and the results were expressed as mg of gallic acid equivalence

(GAE) per g of sample. All experiments were performed in triplicate.

DPPH Free Radical Scavenging Activity

The total free radical scavenging capacity was determined and compared to that of trolox according to the method described by Hsu et al. [9]. The different volume of extracts (50, 100 and 150 µL), was mixed with 1.9 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution. The mixture was shaken vigorously and left for 30 min at room temperature, and the absorbance was then measured at 517 nm against a blank.

The percentage scavenging effect was calculated as Scavenging rate = 100, where A_o was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract. The free radical scavenging activity of extracts was expressed as micromoles of Trolox equivalent antioxidant capacity (TEAC)/g from triplicate extracts using the calibration curve of Trolox. Linearity range of the calibration curve was 20–1000 mM.

Production of Natural Chewing Gum Using Kenger Gum as a Gum Base

First, natural Kenger gum was softened by only performing physical treatment (Fig. 2) without using any chemical. The gum was heated to 140 °C and maintained at this temperature until foamy structure was obtained. Then, the foamed Kenger gum was mixed manually or by mixer for 5 min. After softening, Kenger gum was used instead of artificial gum base in the production of chewing gum. For production of natural chewing gum, softened Kenger gum (25%), glucose syrup (20%), powdered sugar (53%), glycerin (1%), lecithin (0.5%) and sorbitol (0.5%) were mixed for 10 min at 50 °C. After homogenization of the mixture, moulded and shaped natural chewing gum were maintained at room temperature prior to analyses.

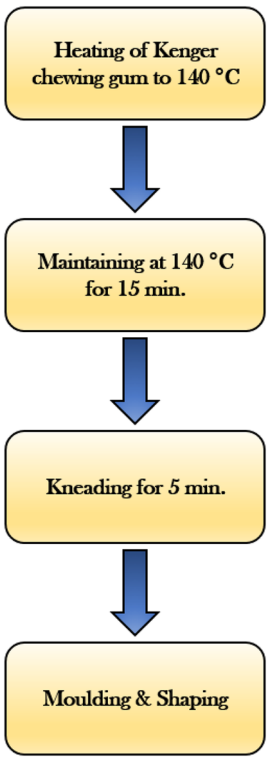

Fig. 2 Softening procedure of Kenger chewing gum

Physical Properties of Chewing Gums

Microstructural Properties

Scanning electron microscopy (SEM) was used to determine microstructure of the samples. For the analyses, the latex samples were mounted on aluminum stubs, sputter coated with 20-nm gold particles, and examined using a Shimadzu SSX 550 SEM (Shimadzu Corporation, Tokyo, Japan), operating at 12 kV. Three replicates were prepared from each of the lyophilized gum samples.

Textural Analysis

Textural properties of the samples were determined using texture analyser (Stable Micro systems, TA.HD Plus) equipped with 5 kg load cell. TPA test was conducted to determine textural properties of chewing gums. P/2 probe (2 mm diameter) was used for the analysis. Pre-test, test and post-test speeds were adjusted to 1, 5 and 5 mm/s respectively. The samples were compressed twice 1 cm inside the samples to calculate textural parameters.

Statistical Analyses

One way ANOVA was performed using SPSS to determine significant differences among textural properties of commercial and Kenger-based chewing gums.

Results and Discussion

Chemical Composition and Functional Properties of Untreated Kenger Gum

Chemical Compositions

There are some previous studies on Kenger plant. However most of these studies are on investigation of functional properties [10–13], using properties as feed [14], and drying kinetics [15] of various parts of Kenger. There is not any study related with compositional properties of Kenger gum.

In this study, some parameters such as trace elements, crude protein content, moisture and ash content, which are important for gum base production, were determined in Kenger gum samples.

Na, Mg, K, Ca, P, Fe, Cu, B, Mn, Zn, Ni, Pb, Cd, Cr, S, Se, Co, Mo, As, Hg, and Al contents were determined as shown in Table 1. For different trace elements, the limit of detection (LOD) ranged from 0.485 µg/kg to 2.85 mg/kg, and the limit of quantification (LOQ) ranged from 10.0 µg/kg to 5.00 mg/kg. Chewing gum is not directly eaten but some toxic metals may be taken to body during chewing. For this aspect, the monitoring of the trace elements, especially toxic metals, in chewing gum is important and very strictly controlled [16]. For the Kenger gum samples, toxic elements, Pb, Hg, As, and Cd were not found. Calcium, which is important for tooth health, is an essential mineral for a variety of physiological and biochemical functions [17]. Calcium content of Kenger chewing gum (845.5 mg/kg) was considered as remarkable.

Table 1 Chemical composition of untreated Kenger chewing gum

Property	Amount
Water	34.6%
Ash	2.0%
Nitrogen	0.065%
Na	102.4 ppm
Mg	339.9 ppm
K	128.8 ppm
Ca	845.5 ppm
P	574.5 ppm
Fe	87.10 ppm
Cu	3.80 ppm
B	2.80 ppm
Mn	3.5 ppm
Zn	11.0 ppm
Ni	1.80 ppm
Cr	0.356 ppm
S	299.3 ppm
Al	133.5 ppm

In a previous study, effect of maturity level on crude protein and ash and dry matter content of Kenger plant was investigated by Kamalak et al. [14] and these parameters were found to be 5.74–14.49%, 11.32–12.54%, and 5.74–14.49% (m/m), respectively. However, for the gum, which is produced by solidification of milk flowing as a result of cutting root of the plant, these parameters was shown significant differences according to our results (Table 1). These findings were not considered as a disadvantage for the production of natural chewing gum.

Functional Properties

The results of the antibacterial activities of methanol, acetone and aqueous extracts of Kenger gum were given in Table 2. As expected, the control treatment (methanol) had no inhibitory effect on any of the test bacteria. It was observed that 80% methanol and acetone extracts had antibacterial activity against all tested strains but 80% methanol extract was the most effective extract with the highest inhibition zones. On the other hand, 50% methanol and acetone extracts from Kenger gum showed antibacterial effect against only one strain, which were *E. coli* O157:H7 ATCC 33150 and *V. parahaemolyticus* ATCC 17802, respectively. In addition, aqueous extract of Kenger had no inhibitory effects on the bacteria. As shown in Table 2, 80% methanol extract showed good antibacterial activity against especially *E. coli* O157:H7 ATCC 33150 (17.97 mm) and *S*. Enteritis ATCC 13076 (15.44 mm) which are Gram negative bacteria.

The results of the total phenolic content and DPPH radical-scavenging activity of Kenger gum extracts were given in Table 3. The values of total phenolic varied between 55.6 and 261.9 GAE mg/100 g. The methanol and acetone extracts contained considerable quantity of phenolic substances. The results indicated that 50% acetonic extract had the highest total phenolic contents (261.9 GAE mg/100 g). This behavior was probably due to higher capacity of 50%

Table 2 Antimicrobial activity of water, methanol and acetone extracts of kenger gum on the pathogen microorganisms

Bacteria	Inhibition zone (mm)					
	Aqueous extract	50% methanolic extract	50% acetonic extract	80% methanolic extract	80% acetonic extract	Negative control (methanol)
Escherichia coli O157:H7 ATCC 33150	-	9.47	-	17.97	13.16	-
Listeria monocytogenes ATCC 7644	-	-	-	12.16	10.70	-
S. Enteritis ATCC 13076	-	-	-	15.44	10.11	-
Staphylococcus aureus ATCC 2592	-	-	-	12.07	10.34	-
Vibrio parahaemolyticus ATCC 17802	-	-	11.22	12.12	15.27	-

(-): No inhibiton zone

Table 3 Total phenolic content and DPPH radical-scavenging activity of water

Samples	Total phenolic content (GAE mg/100 g gum)	TEAC DPPH µmol trolox/g
Aqueous extract	55.6	0.32
Methanolic extract (50%)	159.0	0.65
Acetonic extract (50%)	261.9	0.68
Methanolic extract (80%)	195.6	0.72
Acetonic extract (80%)	191.7	0.71

Methanol and acetone extracts of Kenger gum

TEAC Trolox equivalent antioxidant capacity

GAE Gallic acid equivalent

acetone to solubilize flavonoid components from the Kenger gum detected by the Folin–Ciocalteu method.

The antioxidant activities of Kenger extracts were determined between 0.32 and 0.72 µmol TEAC/g (Table 3). The extracts of Kenger gum studied had potent free radical scavenging activities. The antioxidant activity shown by the Kenger gum might be due to the presence of various flavonoids and phenolic acids. The results showed that methanol and acetone extracts of Kenger gum displayed similar scavenging activities, whereas aqueous extract of Kenger had the lowest antioxidant activity. The 80% methanol extract of Kenger had the highest antioxidant activity (0.72 µmol TE/g) compared with the other extracts. Kenger gum is a kind of a plant polymer (natural latex) which coagulates on exposure to air. It was known that plant latex generally had a wide diversity of bioactive chemicals showing different biological activities such as anti-carcinogenic, anti-proliferative, anti-inflammatory, vasodilatory, antioxidant, antimicrobial, antiparasitic and insecticidal [18]. Topcuoglu et al. [19] remarkably found that Kenger gum had also particular and prolonged antibacterial activity against *Streptococcus mutans* and salivary mutans *streptococci* which were responsible for tooth decay.

Physical Properties of Chewing Gums

The images of the produced chewing gums from Kenger gum without using gum base were shown in Fig. 3. Microstructural properties of untreated and softened Kenger gums and textural properties of these chewing gums and commercial chewing gums were mentioned in this part.

Microstructure Properties of Samples

The outer topographies of Kenger chewing gum samples were assessed by SEM (Figs. 4, 5). Micrographs of treated and untreated samples confirmed textural alteration after heat treatment. The SEM images clearly indicated that treated samples had smoother surface compared to untreated

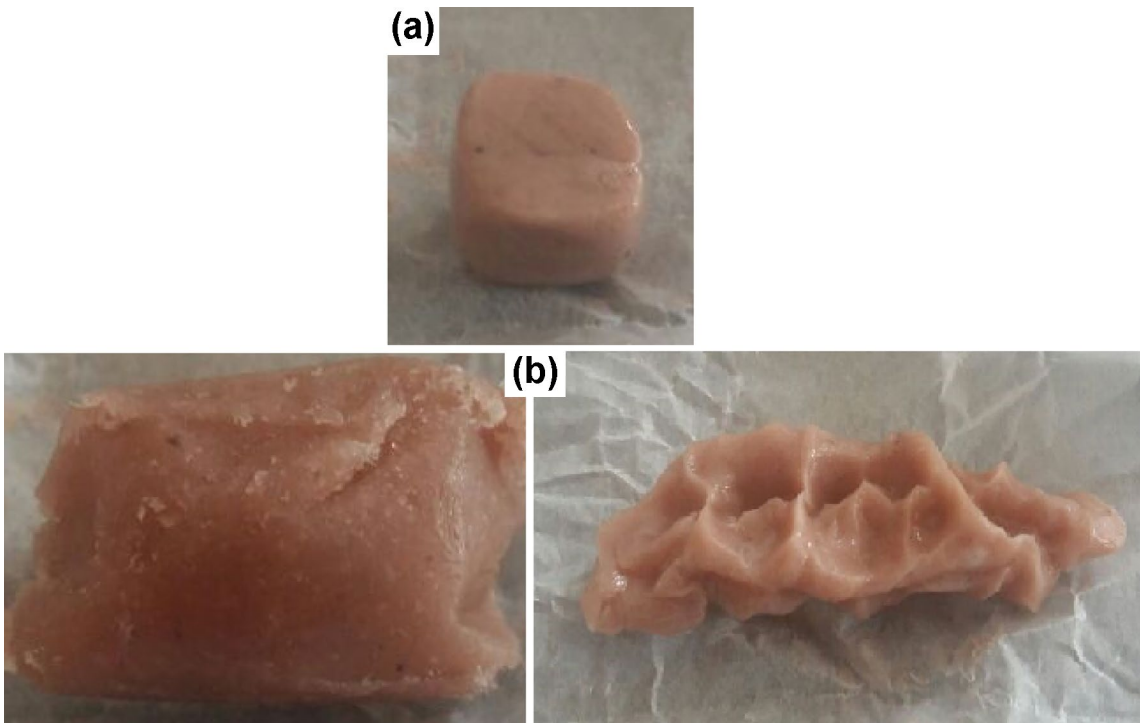

Fig. 3 Photos belonging to chewing gums: **a** Softened Kenger chewing gum, **b** Natural chewing gum produced from Kenger chewing gum used as a natural gum base

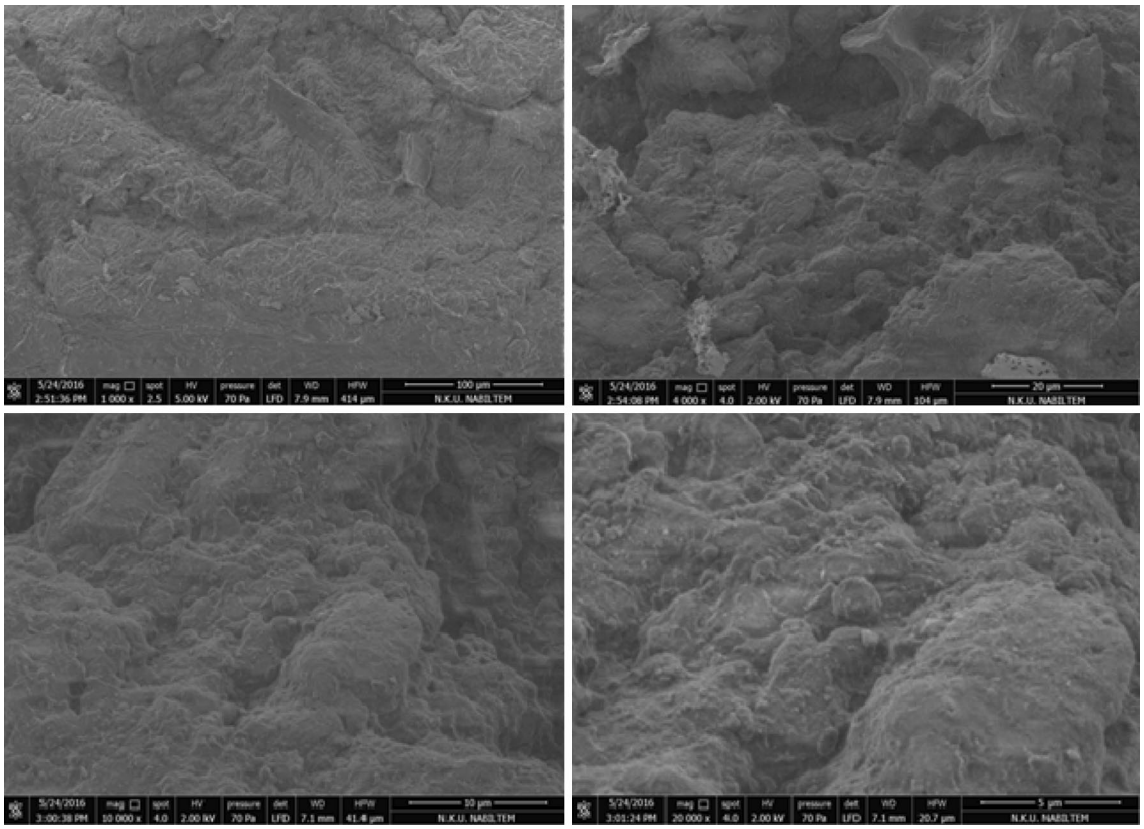

Fig. 4 SEM images of untreated Kenger chewing gum

samples. Therefore, it was concluded that applying the softening procedure, consumer acceptance could be increased due to aesthetic surface property [20].

By the treatment, Kenger gum obtained surface properties similar to starch confections which had continuous and compact gel structure with less pores [21] and more slits. Also there was an increase in surface areas. These structural properties might have some advantages for enhancing Kenger chewing gum by adding some bioactive compounds. Because according to some previous studies, foodstuffs, surface topography of which had slits and higher surface area, might protect the entrapped molecules from exposure to heat and oxygen [22].

Textural Properties

One of the main scientific tests to assess physical properties of chewing gums was texture analysis. However, it was still unclear which parameter(s) in texture data was the most relevant for evaluating good mastication feeling for chewing gums. Table 4 and Fig. 6 showed the effect of softening process on textural parameters of unchewed Kenger gum. As can be seen all textural parameters decreased significantly when the softening process applied which indicated the success of the softening process by improving texture for convenient chewing.

In our preliminary study, it was observed that conducting texture analysis to unchewed gums gave misleading results. For instance, hardness value of commercial chewing gum (Brand 3) was 2120 g when it was analyzed without chewing. This value was much higher than the hardness value of untreated Kenger gum. Therefore, initial chew of this product was harder than untreated Kenger gum although chewing feeling of it was much better than untreated Kenger gum. However, after chewing both Brand 3 and untreated Kenger gum for 3 min before analysis, hardness values became 195.7 and 616.8 g for these products, respectively as could be seen in Table 5. Therefore, there is an "initial chew" term for chewing gums which should be overcome by chewing it before texture analysis. In this study, also the way of chewing and time of chewing after 3 min were also found insignificant (data not shown) by conducting texture analysis on chewing gums chewed by five different volunteers. Hardness values were changed between ±20 g. Although there was not any reference study in the literature, the work of Paradkar et al. [20] could be used as a confirmatory study for our findings. In this study, in-vivo drug release profile was investigated by analyzing the samples chewed by six

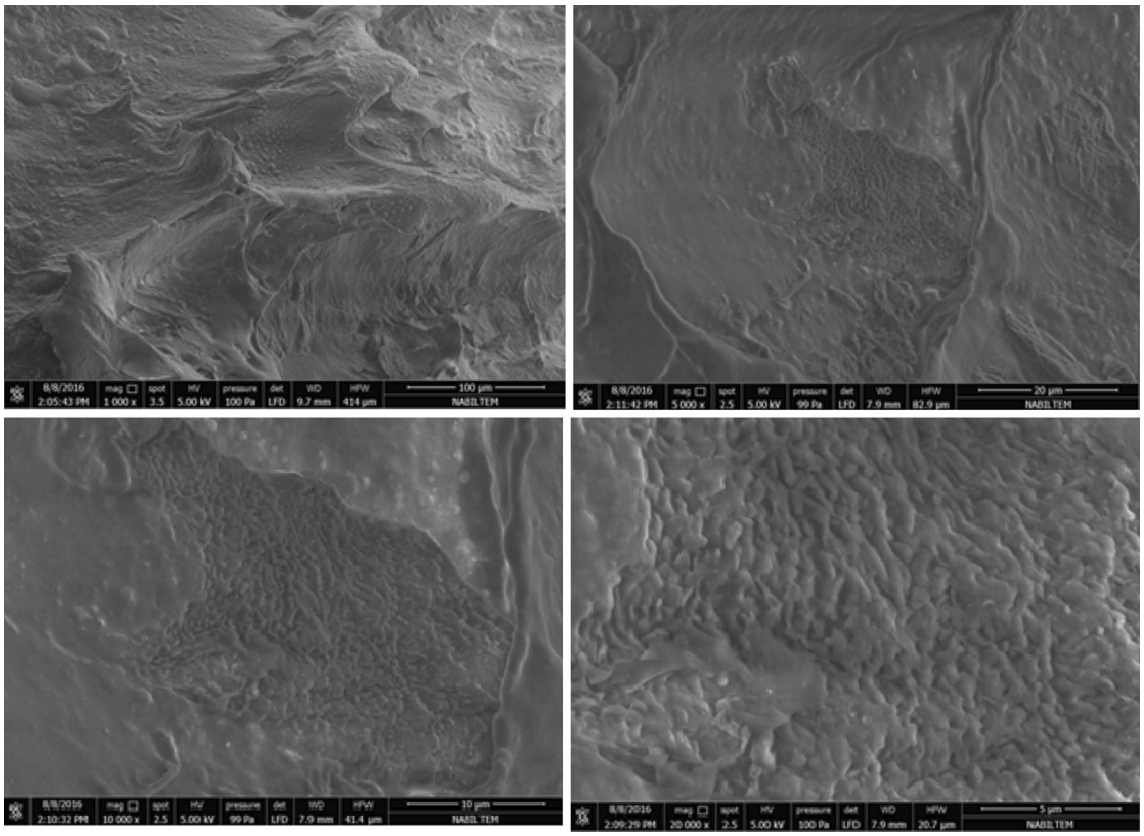

Fig. 5 SEM images of softened Kenger chewing gum

Table 4 Textural properties of unchewed Kenger and softened Kenger chewing gums

Samples	Hardness (g)	Adhesiveness (g s)	Springiness (mm)	Cohesiveness	Chewiness (g × mm)	Resilience
Untreated Kenger	864.7 ± 136.1	−51.9 ± 4.3	0.782 ± 0.031	0.600 ± 0.013	404.9 ± 56.1	0.111 ± 0.008
Softened Kenger	238.6 ± 18.6	−24.2 ± 2.3	0.686 ± 0.051	0.192 ± 0.02	31.4 ± 1.7	0.017 ± 0.001

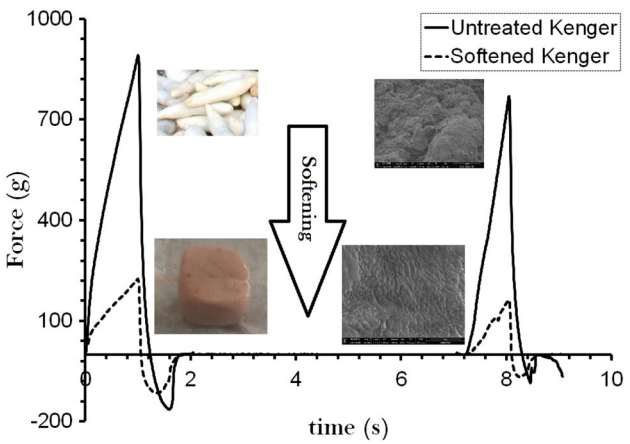

Fig. 6 Effect of softening on textural and microstructural properties of Kenger

panelists for different time periods from 0.5 to 15 min. After analyzing the chewed out samples, there were no significant differences among the panelists regarding the drug release indicating that the way of chewing did not affect the release behavior and indirectly textural properties of the chewing gums. However, the structure of chewing gum can be changed chemically such as when using acidic aromas or using chewing gum immediately after eating anything by means of interaction with chewing gum and residual food particles in mouth.

Therefore, chew out study was conducted to test texture parameters of Kenger gums and four commercial gums for comparison as shown in Table 5 and Fig. 7. Remarkable information was obtained about determining the most important texture parameters for chewing gums. Cohesiveness, springiness and adhesiveness parameters from texture

Table 5 Textural properties of chewed Kenger chewing gums and commercial chewing gums

Samples	Hardness (g)	Adhesiveness (g s)	Springiness (mm)	Cohesiveness	Chewiness (g × mm)	Resilience
Untreated Kenger	616.8 ± 58.3^{a}	-6.01 ± 2.3^{d}	0.789 ± 0.042^{b}	0.782 ± 0.020^{b}	380.0 ± 6.0^{a}	0.247 ± 0.022^{a}
Softened Kenger	160.3 ± 2.3^{cd}	-34.2 ± 1.3^{c}	0.806 ± 0.052^{b}	0.484 ± 0.005^{d}	62.5 ± 4.3^{d}	0.030 ± 0.000^{c}
Kenger with sugar	256.1 ± 46.0^{b}	-79.4 ± 13.0^{b}	0.787 ± 0.010^{b}	0.493 ± 0.065^{d}	100.7 ± 32.2^{c}	0.032 ± 0.001^{c}
Brand 1	70.7 ± 9.7^{e}	-64.5 ± 3.4^{b}	0.990 ± 0.001^{a}	0.971 ± 0.048^{a}	67.7 ± 6.0^{d}	0.040 ± 0.001^{bc}
Brand 2	87.1 ± 12.4^{de}	-15.5 ± 3.0^{d}	0.799 ± 0.035^{b}	0.608 ± 0.018^{c}	42.1 ± 2.9^{d}	0.047 ± 0.003^{bc}
Brand 3	195.7 ± 18.1^{bc}	-97.4 ± 9.0^{a}	0.995 ± 0.006^{a}	0.882 ± 0.082^{ab}	170.9 ± 0.2^{b}	0.059 ± 0.007^{b}
Brand 4	114.0 ± 15.1^{de}	-72.4 ± 7.5^{b}	0.990 ± 0.001^{a}	0.917 ± 0.042^{a}	103.2 ± 9.0^{c}	0.040 ± 0.000^{bc}

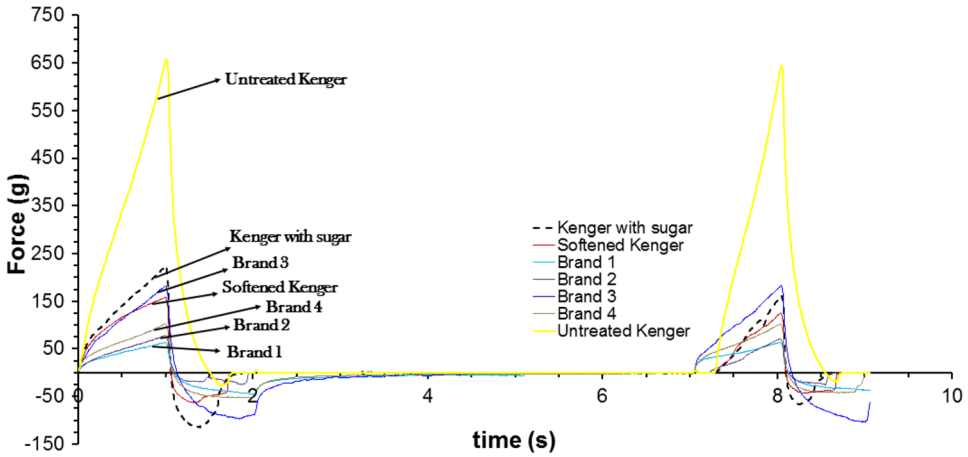

Fig. 7 Textural properties of chewed chewing gums

analysis were found to have no direct effect on revealing chewing gums with good masticatory properties since similar values were obtained for untreated Kenger gum and the other gums. Concerning the hardness value, significant decrease was observed for Kenger gum after softening treatment. It was observed that chewing gum with best chewing property should have hardness value between 60 and 300 g. However, products that do not exhibit chewable property also have a hardness value between these values. For instance, Habilla and Cheng [23] studied the texture of jelly candy samples prepared from different thickening agents and found that hardness values of the samples changed between 125 and 225 g. Therefore, using hardness value was also insufficient to find good chewing gums with desired chewiness properties. The other texture parameter which had no relevance to determine good quality chewing gums was chewiness since chewiness is the product of springiness, cohesiveness and hardness values all of which were found ineffective.

Nevertheless, resilience parameter was observed to be the best parameter to determine chewing gums having the best sensorial chewiness property. As shown in Table 5, resilience value of Kenger gum substantially decreased from 0.247 to 0.030 after softening treatment. Resilience values of commercial chewing gums and treated Kenger gums changed between 0.030 and 0.059 which could be the range of chewing gums with desired chewiness properties. This finding was preliminary and by texture analysis new parameters can be found which can describe the best sensorial chewing properties for chewing gums but it was not the scope of this work. Future studies should focus on this important subject as a bridge between sensory and texture properties is still unexplored.

Conclusions

Environmental problem posed by commercial chewing gums is an important concern worldwide. Also, functional and natural foods are gradually known and preferred by consumers. Natural chewing gum having these properties with desirable textural properties was obtained from Kenger plant (*G. tournefortii*). Thermal processing was used to soften traditionally used Kenger chewing gum. SEM images validated softness of the natural chewing gum with more plain structure. Locally torn surfaces might be advantageous for carrying bioactive compounds. Texture properties of produced natural chewing gum were compared with commercial chewing gums and similar values were obtained. Resilience value was found to be the most relevant parameter to choose

chewing gums with desired chewing properties. Amount of calcium was the highest among other elements existed in the Kenger gum which showed the importance for teeth. Observed antioxidant and antibacterial effects of natural polymer demonstrated the benefits of using Kenger gum in chewing gum production for both health and environment.

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