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Impact of cooking on nutritional contents of kenger in terms of antioxidants

Indrani Kalkan^{1*}, Ozan Emre Eyupoglu², Sukru Karatas³, Zakia El Miri Aissaoui⁴ and Rusen Anik⁵

Abstract

Kenger (*Gundelia tournefortii*) grown in Sanliurfa and neighbouring provinces in Turkiye, is known for its health promoting potential due to the presence of bioactive compounds as phenolics and antioxidant vitamins. However, such compounds in food may be affected by thermal treatment as cooking. This study investigated the impact of common cooking techniques (boiling and stir-frying in oil) on phenolics, vitamin C content and antioxidant activity of kenger. Phenolic compounds were analysed using LC–MS/MS and DPPH inhibition. Vitamin C was quantified using HPLC and antioxidant activities were assessed by DPPH inhibition, ABTS and FRAP methods. Major phenolic compounds in raw samples were vanillic ($18.755 \pm 0.606 \mu\text{g/g}$) and fumaric ($16.211 \pm 0.524 \mu\text{g/g}$) acids. In boiled kenger, significant loss of fumaric ($5.789 \pm 0.187 \mu\text{g/g}$), in stir-fried kenger, loss of vanillic ($15.604 \pm 0.504 \mu\text{g/g}$) and fumaric ($8.113 \pm 0.262 \mu\text{g/g}$) acids were noted ($p < 0.05$). Vitamin C content of raw kenger was $7.104 \pm 0.074 \mu\text{g/g}$ but decreased to $6.812 \pm 0.22 \mu\text{g/g}$ in boiled and $6.898 \pm 0.072 \mu\text{g/g}$ in stir-fried samples. The radical scavenging potentials of sample extracts at different concentrations (25 mg/mL, 50 mg/mL, 75 mg/mL, 100 mg/mL, 150 mg/mL and 300 mg/mL) were tested by DPPH, FRAP and ABTS methods. At 300 mg/mL, antioxidant activity in raw kenger was calculated as $1.350 \pm 0.0007 \mu\text{moles/mg/mL}$ (FRAP), $0.731 \pm 0.0008 \mu\text{g}$ (EC_{50} DPPH) and $52 \pm 3.45\%$ TEAC (ABTS). In general, antioxidant activities for all methods increased with rising concentration of the sample extract. Antioxidant activity of boiled samples decreased significantly as per FRAP and increased for stir-fried kenger at higher sample concentrations as per FRAP and ABTS, possibly due to chemical changes during high thermal treatment in oil. Correlation between cooking methods was not significant ($p > 0.05$) in terms of phenolic compounds and vitamin C. Correlation between antioxidant activity assessing methods were only significant for 75 and 100 mg/mL sample concentration ($\rho = -0.90$ $p < 0.05$). In conclusion, boiled and stir-fried kenger is suggested as optional consumption methods. Inclusion of condiments, herbs, olive oil, yoghurt during service would not only increase palatability but also add to the health benefits.

Keywords Antioxidant activity, Kenger, Boiling, Stir-frying, Phenolic compounds, Vitamin C

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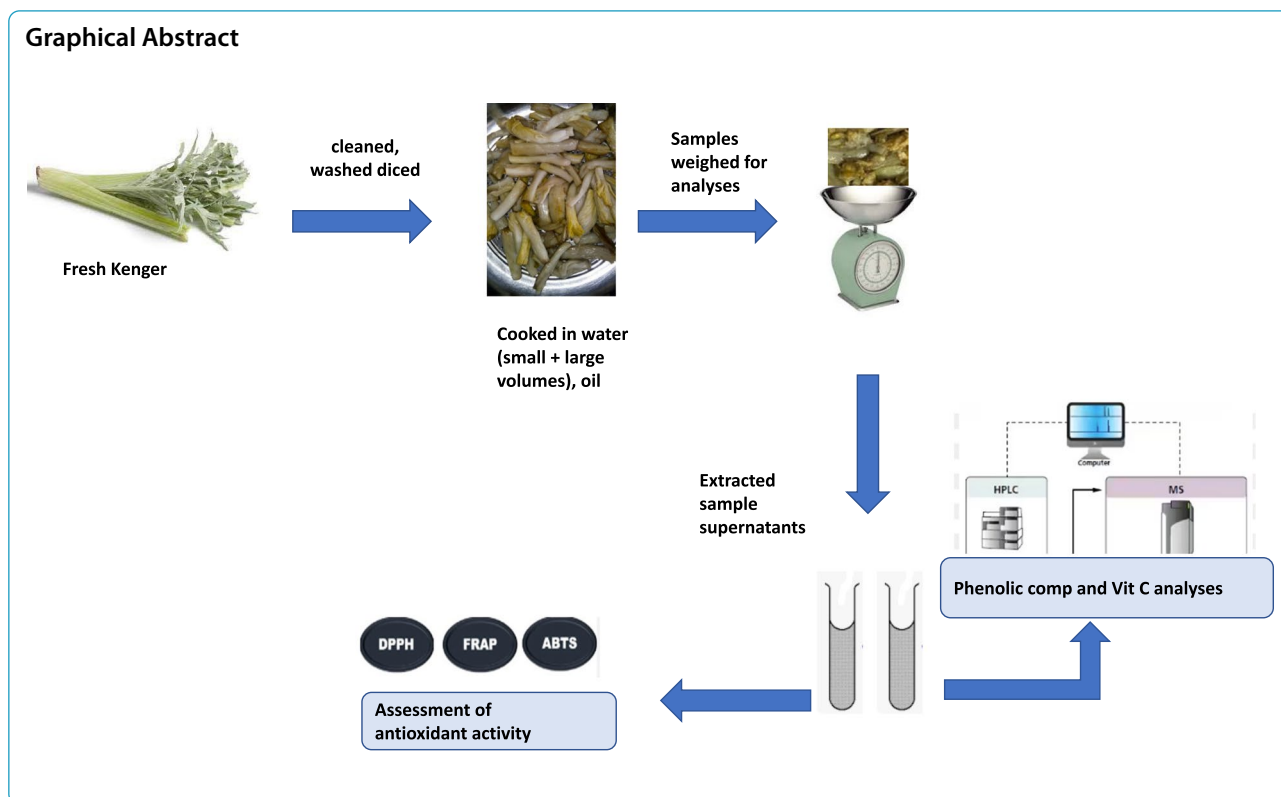
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Introduction

Gundelia belonging to the Asteraceae family is a thorny perennial herbaceous plant of medium height (20–100 cm) with latex and compound inflorescence comprising of reddish-purple florets which can be found from February to May. Kenger thistle (*Gundelia tournefortii*) resembling artichokes, belongs to the same family, and grows in a number of countries in the Mediterranean region with a semi-desert type climate (Gustaf, 2013). Particularly in Urfa and Tunceli south east of Turkiye, *G. tournefortii*, or 'kenger' is widely cultivated and harvested by local people and is considered very valuable due to its well-known health benefits (Cakilcioglu, 2020; Ozel & Kosar, 2017). In the early part of the year, kenger plants growing in the wild are collected, chopped at the base and the thorns are removed. The undeveloped flower heads, stems, leaves and roots of kenger plant is widely consumed (Gustaf, 2013). Particularly, the fresh tender leaves of *Gundelia* are used to make soup in Urfa region of Turkiye. Kenger is consumed boiling in water or stir-fried in vegetable oil, mixed with eggs, meat balls or stewed meat and served with yogurt, herbs and condiments as chilli flakes, tomato puree, garlic paste, black pepper and salt (Cakilcioglu, 2020; Ozel & Kosar, 2017). Kenger extract has been reported to be used in the fermentation of milk, in

ice cream as a stabilizing agent and for increasing consistency in yogurt (Demir, 2013; Say & Guzeler, 2016).

Food grade chewing gum (kenger sakizi in Turkish) is made from the latex of *Gundelia*, a fact that was mentioned by Tournefort long back in 1718. The flower heads and roots of kenger is roasted and ground to be consumed as coffee, (kenger kahvesi in Turkish). In recent times, mature seeds of kenger have been used to extract oil (kenger yagi in Turkish) also known for health benefits. Remains of charred *Gundelia* inflorescences found in Turkiye and Iraq from the neolithic period indicate that the oil was extracted from *Gundelia* seeds 10,000 years ago (Gustaf, 2013; Karaaslan et al., 2014). *Gundelia* seeds (sissi in Northern Iraq) are roasted, salted—has a taste close to sun-flower seeds and widely consumed in this area (Gustaf, 2013). Kenger (*Gundelia tournefortii*) is known for its bioactive components and health properties. Presence of caffeoylquinic acid derivatives (cinaren and chlorogenic acid), phenolic compounds (vanillic acid, fumaric acid, gallic acid) and other active ingredients (limonene, zingiberene, saponins) contribute to the plant's biological activity (Haghi et al., 2011; Hajizadeh-Sharafabad et al., 2016). Kenger has been reported to be consumed for amelioration of symptoms in diabetes, liver diseases, indigestion, cramps, bronchitis, mumps, stomach pain, diarrhoea, mouth sores, migraine, heart disease,

stroke, stomach pain, vitiligo, strengthening nerves, and purification of blood (Asadi-Samani et al., 2013; Azeez & Kheder, 2012). In addition, certain bio active compounds as sitosterol, stigmasterol, lupeol, gitoxygenin, α -amyrin and artemisinin, present in *G. tournefortii*, acting synergistic, have been suggested to have anti-carcinogenic and preventive roles (Abu-Lafi et al., 2019).

Although kenger may be consumed raw, quick cooking methods like steaming, stir-frying in vegetable oil and boiling can be preferred not only to increase the palatability but also increase the nutritional value when cooked with meat, eggs, spices and served with yogurt or whole grains. It has long been perceived that bio-active compounds in plants and food products are affected by pedoclimatic conditions, agronomic factors, harvesting methods, storage conditions and processing/cooking techniques (Fombang et al., 2021; Kalkan & Yucesan, 2013). In this respect, it would be significant to understand the effect of common cooking methods on the bio-active components of kenger.

The objective of this study was to determine the effect of boiling and stir-frying (in oil) on phenolic compounds, vitamin C content and antioxidant properties of kenger plant samples. Findings of this study would guide consumers to choose appropriate cooking methods for kenger that would be palatable and also be beneficial to their health.

Materials and methods

Plant materials

Fresh kenger used for research material was purchased from several open markets, downtown, Sanliurfa during months of March–April, which is considered the peak harvest season (February to April) for kenger in this area. Kenger plants were obtained from three different sellers (1 kg from each) who were randomly chosen. Obtained kenger plants were mixed thoroughly and 1 kg was separated out for analyses. Plant material was washed with tap water, dried on paper towels and non-edible thorny edges were removed. Cleaned kenger stems were cut into pieces each weighing approximately 3–4 g. Three portions, each weighing 200 g were separated out. One portion was retained as raw, the second was boiled in water and the third, stir-fried in oil. All cooking methods were repeated thrice (at three different time intervals). Cooked samples were weighed to determine the weight loss during cooking. Samples were used in triplicate, for each analysis.

Cooking by boiling in small amounts of water (B)

200 g of kenger sample was added to a stainless-steel pot (Tefal branded 1 L, with double base) with a diameter of 12–14 cm. Approximately 100 ml of boiling water

was poured into the pot and brought to boil. The pot was then covered and boiling was continued for 10 min at the minimum flame (Arcelik branded, standard kitchen stove, with natural gas connection). The cooked sample was brought to room temperature prior to analyses.

Stir-frying in oil (F)

15 ml (one table spoon) of sunflower oil was added to a fry-pan with a diameter of 32 cm (Tefal thermospot, 32 cm), heated to reach a temperature of approximately 190°C. The kenger sample (200 g of chopped kenger branches) was added and stir-fried for 5 min at medium flame (Arcelik branded, standard kitchen stove, with natural gas connection). The stir-fried sample was brought to room temperature prior to analyses.

Dry weight determination (DW)

Because of varying water content in raw and cooked vegetables, all calculations were based on their dry weight basis. The raw and cooked samples were blended in a domestic kitchen blender (Tefal master chop Glass Blender 500 W) for 1 min. For determination of dry matter, 2–3 g weighed homogenized samples (in triplicate) were taken, and smeared in a thin layer on the surface of a porcelain petri-dish used for drying. The samples were put to dry in a convection oven at $100^{\circ} \pm 2^{\circ}\text{C}$ until a constant weight was reached (Kalkan & Yucesan, 2013).

Extraction of kenger sample for analysis

Extraction was done in distilled water and methanol for analysis. Water and methanol are highly polar in nature. Polyphenols being polar in nature is better extracted by polar solvents. In addition methanol being acidic, is advantageous in high extraction yields of antioxidants from food samples. Although both reagents are used for DPPH as well as FRAP, some studies suggest methanol for DPPH and water for FRAP. On the other hand, a mixture of both (methanol 80:20 water) has also been suggested for extraction of phenolic compounds in food materials (Namvari et al., 2017). To prepare the sample extracts, 300 mg of the raw/cooked samples were weighed (Shimadzu BL-320H) and placed into 5 ml eppendorf tubes. 2 ml of distilled water (Electromag M4 water distillator) and methanol (99.6% Sigma-Aldrich) was added to the sample and homogenized with couple of metal homogenizing beads in a homogenizer (Beadblaster 24 tube homogenizer) for 45 min at 4°C. The samples were then centrifuged at 15000 rpm (Eppendorf Centrifuge 5417 C) for 10 min. The supernatants were collected in separate 2

ml Eppendorf tubes and stored at -80°C until use (Mata et al., 2007).

Analysis of phenolic compounds by LC–MS/MS (Liquid Chromatography Tandem Mass / Mass Spectrometer System)

LC–MS/MS analyses of phenolic compounds present in the samples were performed using Nexera LC-30AD dual pressure, DGU-20A3R degasser (methane, H_2S , CO_2 formed in the mud used in oil drilling gas purifying device) CTO-10ASvp column cooker and SIL-30AC sampler equipped with ODS4 chromatographic separation column reserved on C_{18} . The temperature of the column was stabilised at 40°C (Ertas et al., 2021). The elution gradient was composed of eluent A (water+5 mM ammonium formate+0.1% formic acid) and eluent B (methanol+5 mM ammonium formate+0.1% formic acid). During first 25 min, flow rate of eluent B was adjusted from 20–100% complemented by eluent A. During the next 10 min, 100% of eluent B was given. The complete eluent flow rate was settled as 0.5 mL/min and 5 μL , respectively. For the mass spectra, the electron source nebulizer gas pressure was set at 40 psi with a flow rate of 7 L/min at 350°C , while the sheath gas was set to 9 L/min at 350°C . The capillary was set at 3600 V. The spectra were recorded in positive and negative ionization modes. The MS spectrum of the detected compounds was recorded from m/z between 100 to 1200. Quantitative data for phenolic compounds were obtained from calibration curves constructed using the standards. A total of 150 fingerprints compounds were collected from the methanolic extracts of Kenger in each scan modes (positive and negative). Resolution of the positive scan mode reduced the phenolic compounds to 25 compounds by means of multiple resolution mode (MRM) based chromatogram analysis. Shimadzu LC–MS 8030 model was used for LC–MS/MS analysis with negative ionising modulus of ESI source in spectrometer. LC–MS/MS data were evaluated with Lab Solutions software. MRM (multiple reaction monitoring) mode of analyses was used for evaluation: For the analysis of the components, quantitative analyses of each were conducted thrice, for validation of the results (Ertas et al., 2021). In this study, 25 phenolic components in kenger were assessed, since the column used was specific for the detection of phenolic compounds only (C_{18} column Hypersil Gold, 100 mm \times 2.1 mm i.d., 3 μm , Thermo Fisher Scientific, USA).

Vitamin C analysis by HPLC

High performance liquid chromatography (HPLC) was used for vitamin C analysis in raw and cooked kenger samples. HPLC is a chromatographic technique, based on

the distribution of substances between the mobile phase and the stationary phase under high pressure, where the mobile phase is liquid (Bengu, 2014). Shimadzu (Japan) LC-20AT chromatography device, SIL-20A HT autosampler, CTO-10AS column furnace and SPD20A UV detector were used for analyses. Mobile phase (isochrotic system) was comprised of 0.1% formic acid, methanol and water (v/v, 85:15), the column flow rate was 1 mL/min and injection volume was 10 μL . The column (GL Sciences brand ODS-3, 25 mm \times 4.6 x 3 μm) temperature was maintained at 25°C and detection wavelength was set at 245 nm. The LC parameters for the LC-UV–MS method were selected after screening several columns and solvent combinations with different isocratic profiles. ESI negative ion mode provided the best response, where the unprotonated molecular ion $[\text{M}-\text{H}]^{-}$ at m/z 175 was monitored well on the MS chromatograms. Retention times of vitamin C were $t_R=5.891$ (UV) and 6.069 min (MRM). Each sample was studied thrice and mean value was recorded (Bengu, 2014).

Detection of antioxidant activity (aa)

FRAP (Ferric ion reducing antioxidant power) analysis

FRAP analysis involves a redox-linked colorimetric reaction based on the principle of determination of antioxidants in a sample through its reduction capacity of Ferric (III) to Ferrous (II) ion. When preparing the FRAP solution, firstly 300 mM concentration pH: 3.6 Acetate Buffer solution was prepared. 1.555 g Sodium Acetate and 8 mL Glacial Acetic Acid was added and the solution was prepared at room temperature. More than 0.73 mL into 500 mL of water with 40 mM concentration at room temperature. For TPTZ 10 mM solution, 0.031 g TPTZ was added to 10 ml 40 Mm HCl and distilled water is added and 50°C water bath ready to use was brought in. For Ferric Chloride 20 mM solution, dissolve 0.054 g $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ was prepared by adding 10 mL distilled water. 200 ml acetate buffer, 20 mL TPTZ, was mixed and kept in 24 mL distilled water at 37°C water bath for FRAP reagent.

For analysis, 1 mL FRAP solution was added to 30 μL of plant extract and then the mixture was shaken and left in an incubator at 37°C for 4 min. Readings were made at a wavelength of 593 nm (Guo et al., 2003).

DPPH (2,2-Diphenyl-1-Picrilhydrazyl) free radical removal analysis

DPPH free radical removal activity was conducted as per Blois method. The method is based on the use of the stable free radical diphenylpicrylhydrazyl (DPPH) to estimate the activity of antioxidants in the sample, using the parameter EC_{50} (substrate concentration to produce 50% reduction of the DPPH). 100 mM DPPH solution was

used as the free radical. In this method, the DPPH radical combines with the hydrogen of the antioxidant substance to form a single electron-reduced DPPH, while the molar absorption coefficient of the DPPH radical at 517 nm and the colour changes from purple to yellow. A 1 mg/mL stock solution of BHT pure standard antioxidant compound was prepared. 6 different concentrations were obtained by the “half-half” dilution method. Each concentration was treated with 100 mM DPPH solution at a one-to-one ratio, and the absorbance was read at 517 nm after the reaction was left in the dark for 30 min. EC₅₀ value (ug/mL) was determined from the calibration graph drawn. Initial concentrations were determined by reacting to different concentrations of the cooked kenger samples in the same way, with the DPPH radical. EC₅₀ (ug/mL) values were determined from the calibration graphs drawn against the absorbance created with the six dilution concentrations. It was observed that the EC₅₀ (ug/mL) values of Kenger plants cooked differently were very close to the BHT standard EC₅₀ value (Guzel et al., 2013). The reduced absorbance yields the remaining amount of DPPH solution namely the free radical scavenging activity. After incubation for half an hour at room temperature in the dark, the absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH concentration (μM) in the reaction medium was calculated from the following calibration curve, determined by linear regression ($R^2=0.999$).

ABTS⁺ [2,2-Azinobis (3-Ethylbenzothiazoline 6-Sulfonate)] radical scavenging activity method

The ABTS⁺ method recommended by Rice-Evans & Miller was used for this assay (Rice- Evans et al., 1996). This method measures the relative ability of antioxidants to scavenge the ABTS⁺ radical [2,2'-azinobis (3 ethylbenzothiazoline-6-sulphonic acid)] generated in aqueous phase, as compared with a Trolox reagent which is a synthetic antioxidant standard (water soluble vitamin E analogue). The ABTS⁺ radical is generated by reacting with a strong oxidizing agent (e.g. potassium permanganate or potassium persulfate) with the ABTS salt. Prepared stock solution (samples) was diluted with ethanol. For each solvent in different concentrations (20μl, 30μl, 40μl values), antioxidant activities were determined for 3 min at 30 s intervals. Calculations were made taking account of dilution factors. To prepare the ABTS+ solution, 0,15 g of ABTS was dissolved in 100 ml of pure water. For the preparation of the potassium persulfate solution, 0,04 g of potassium persulfate was added to 100 m l of ABTS solution a little at a time. This solution was stirred on a magnetic stirrer for half an hour. The scavenging radical activity method is based on the determination of the

scavenging activity of the ABTS radical, which is a strong radical antioxidant layer. 7.4 mM ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) was dissolved in 1 mL of distilled water and 1 mL of 2.6 mM potassium persulphate was added. This mixture was left in the dark for 12–16 h at room temperature. Then 1 mL of the mixture was taken, and 60 mL of methanol was added, 2850 μL was taken and 150 μL of plant extract was added. This was left in the dark for 2 h. The absorbance value was read at 734 nm with a spectrophotometer. The concentration obtained after calculating the percentage of plant extract and standards was expressed as a result as percentage of ABTS radical scavenging activity.

Statistical evaluation

For statistical calculations, the software program IBM SPSS 20.0 package program was used for analysis of the data. Continuous variable was presented as mean ± standard deviation (SD). Because of the smaller number of samples, data was considered non-parametric. Therefore, one-way analysis of variance (ANOVA) and Kruskal–Wallis test was conducted (significance level $p < 0.05$) to analyse the difference between the contents of phenolic compounds and vitamin C in kenger as a result of the cooking methods applied. The Spearman correlation test was conducted to analyse the correlation between phenolic and vitamin C contents of kenger samples. Also, correlation was studied between antioxidant assessment methods in terms of antioxidant activities at different concentrations of the sample extracts. In addition, Tukey HSD (honest significant difference) test was applied to determine the significance and source of this difference.

Results

Phenolic compounds in raw and cooked kenger samples

Phenolic component analysis of methanol extracts of the Kenger raw and cooked samples were performed by LC–MS/MS technique. Out of the 25 phenolic compounds assessed (catechinhydrate, quercetin, acetohydroxamic acid, vanillic acid, resveratrol, fumaric acid, gallic acid, caffeic acid, fluoridizine dehydrate, oleuropein, hydroxycinnamic acid, ellagic acid, myricetin, silymarin, curcumin, naringenin, kaempferol, salicylic acid, hydroxybenzoic acid, butaine, luteolin, alizarin, thymoquinone, protocatechuic acid ethyl ester, hydroxy 1,4 naphthoquinone), only four were detected in kenger namely vanillic, fumaric, caffeic and hydrobenzoic acid. Fumaric acid was found to be significantly high in raw kenger samples as compared to cooked ones. There was also a significant difference between boiled and stir-fried samples ($p < 0.05$) (Table 1).

Table 1 Phenolic compounds and vitamin C in raw and cooked kenger samples

	RAW KENGER (µg/g) + SD	BOILED KENGER (µg/g) + SD	STIR FRIED KENGER (µg/g) + SD
Vanillic acid	18.755 ± 0.606	18.673 ± 0.604 ^b	15.604 ± 0.504 ^{ab}
Fumaric acid	16.211 ± 0.524 ^a	5.789 ± 0.187 ^{ab}	8.113 ± 0.262 ^{ab}
Caffeic acid	N. D	0.340 ± 0.010 ^{ab}	1.291 ± 0.042 ^{ab}
Hydroxybenzoic acid	0.129 ± 0.004 ^a	0.085 ± 0.002 ^{ab}	0.110 ± 0.003 ^{ab}
Vitamin C	7.104 ± 0.074 ^a	6.812 ± 0.22 ^{ab}	6.898 ± 0.072 ^{ab}

Analysis in each group (n = 3) was calculated as mean ± SD (standard deviation) and statistical differences between the groups were indicated

^a there is a significant difference between raw Kenger and other cooked samples (p < 0.05)

^b there is a significant difference between boiled and stir-fried group (p < 0.05)

Vitamin C contents in raw and cooked kenger samples

High performance liquid chromatography (HPLC) was used for vitamin C analysis in raw and cooked kenger samples. Raw kenger was found to contain the highest level of vitamin C (7.104 ± 0.07 µg/g). Loss of the vitamin was noticed in boiled (6.812 ± 0.22 µg/g) as well as fried (6.898 ± 0.072 µg/g) samples (p < 0.05). The loss was higher in boiled samples as compared to fried ones and the difference was statistically significant (p < 0.05) (Table 1).

The trend in changes in contents of phenolic compounds and vitamin C during cooking methods have been shown in Fig. 1.

Correlation between phenolic compounds and vitamin C contents in samples

Spearman Correlation test was conducted between phenolic compounds and vitamin C contents of raw and cooked kenger samples in order to understand if there was a correlation between these two parameters based on cooking methods. However, no significant correlation

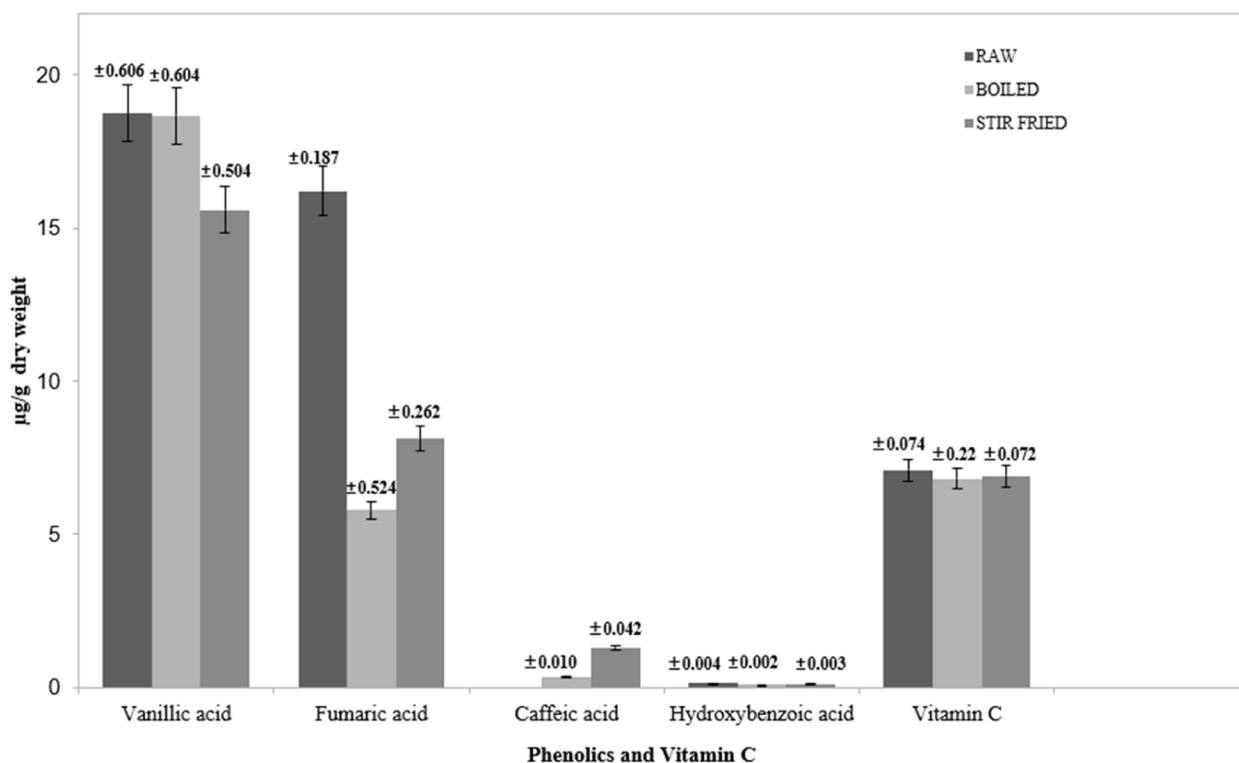


Fig. 1 The effect of cooking methods on phenolics and vitamin C in kenger

was found between the two ($p > 0.05$). The results obtained were at a low level of reliability due to the less number of samples.

Antioxidant activity in raw and cooked kenger samples

FRAP method

Antioxidant activity of raw kenger plant and its two differently cooked (boiled in water and stir-fried in oil) samples were investigated by FRAP method. The samples were extracted in methanol and water at different concentrations (25 mg/mL, 50 mg/mL, 75 mg/mL, 100 mg/mL, 150 mg/mL and 300 mg/mL). FRAP antioxidant activities of water and methanol extracts for different cooking methods were given by proportioning the Trolox equivalent (TE) dry matter concentration. In general, antioxidant activity increased with the concentration of the sample, however there were fluctuations at some of the concentrations (Table 2). The highest antioxidant activity value was found in stir-fried sample in methanol extract with 1.860 ± 0.0006 $\mu\text{mole TE/mg DM/mL}$ at 300 mg/mL concentration followed by 1.350 ± 0.0007 $\mu\text{mole TE/mg DM/mL}$ in raw kenger and 1.048 ± 0.0005 $\mu\text{mole TE/mg DM/mL}$ in stir-fried sample in methanol extract at 150 mg/mL concentration.

DPPH method

Methanol and water extracts of raw and cooked (boiled and stir-fried in oil) samples were used for determination of antioxidant activity by DPPH method. Antioxidant activity was found to be dose dependent, highest activity being at 300 mg/mL concentration. Based on cooking methods examined, the highest antioxidant activity value was found in stir-fried kenger water extract at 300 mg/mL concentration as 1.025 ± 0.0005 $\mu\text{g (EC}_{50}\text{DPPH)}$ followed

by raw kenger having 0.731 ± 0.0008 $\mu\text{g (EC}_{50}\text{DPPH)}$ activity. In general, antioxidant activity increased with the concentration of the sample, however there were fluctuations at some of the concentrations (Table 3).

ABTS method

Methanol and water extracts of raw and cooked (boiled and stir-fried in oil) samples were used for determination of antioxidant activity by ABTS method. Although antioxidant activity was found to be dose dependent, however there were fluctuations in the results at some concentrations of the extracted samples. Based on cooking methods examined, the highest antioxidant activity value was observed in 300 mg/mL solution of boiled kenger extracted in methanol ($90.0 \pm 5.55\%$ TEAC) followed by stir-fried kenger extracted in methanol ($89.8 \pm 6.40\%$ TEAC) and boiled kenger extracted in water ($89.5 \pm 5.52\%$ TEAC) (Table 4).

Correlation between the antioxidant activity assessing methods

As a result of examining the antioxidant activity tests performed with six different concentrations of the extractions of raw and cooked kenger samples in water (dH₂O) and methanol (MeOH) with the Spearman correlation coefficient; it was determined that there was a negative correlation between ABTS and DPPH methods at concentrations of 100 mg/mL and 75 mg/mL ($\rho = -0.90$ $p < 0.05$), no relationship was found between other methods and concentrations (Table 5). The results obtained were at a low level of reliability due to the less number of samples.

Table 2 Antioxidant activity values of raw and cooked kenger samples with increasing concentration of sample: FRAP method ($\mu\text{mole TE/mgDM/mL} \pm \text{SD}$)

Sample Concentration	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	150 mg/mL	300 mg/mL
Raw kenger	0.654 ± 0.0004	0.684 ± 0.0004	0.723 ± 0.0004	0.774 ± 0.0005	0.918 ± 0.0005	1.350 ± 0.0007
Boiled kenger (extracted in methanol)	0.229 ± 0.0001^a	0.287 ± 0.0001^a	0.422 ± 0.0002	0.502 ± 0.0001	0.396 ± 0.0002^c	0.568 ± 0.0003^a
Boiled kenger (extracted in water)	0.233 ± 0.0001	0.278 ± 0.0001	0.191 ± 0.0001^a	0.207 ± 0.0001^a	0.207 ± 0.0001^a	0.674 ± 0.0003
Stir-fried kenger (extracted in methanol)	0.295 ± 0.0001	0.447 ± 0.0002	0.656 ± 0.0002	0.750 ± 0.0003	1.048 ± 0.0005^b	1.860 ± 0.0006^b
Stir-fried kenger (extracted in water)	0.264 ± 0.0001	0.237 ± 0.0001	0.385 ± 0.0001	0.446 ± 0.0001	0.366 ± 0.0002^c	1.039 ± 0.0005

Analysis in each group ($n = 5$) was calculated as mean \pm SD (standard deviation) and statistical differences between the cooked groups

^a significant loss in antioxidant activity as compared to other cooked groups ($p < 0.05$)

^b significant increase in antioxidant activity as compared to other cooked groups ($p < 0.05$)

^c significant decrease in antioxidant activity as compared to its lower sample concentration ($p < 0.05$)

Table 3 Antioxidant activity values of raw and cooked kenger samples with increasing concentration of sample: DPPH method (µg/mL ± SD)

Sample Concentration	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	150 mg/mL	300 mg/mL
Raw kenger	0.626 ± 0.0001	0.625 ± 0.0001	0.549 ± ^c 0.0001	0.482 ± ^c 0.0001	0.522 ± 0.0001	0.731 ± 0.0008
Boiled kenger (extracted in methanol)	0.174 ± 0.001	0.165 ± 0.0001	0.156 ± 0.0001	0.159 ± 0.0001	0.155 ± 0.0003	0.154 ± 0.0001
Boiled kenger (extracted in water)	0.405 ± 0.0002	0.162 ± ^c 0.0002	0.164 ± 0.0003	0.169 ± 0.0002	0.174 ± 0.0002	0.197 ± 0.0003
Stir-fried kenger (extracted in methanol)	0.153 ± ^a 0.0002	0.156 ± ^a 0.0002	0.152 ± ^a 0.0002	0.147 ± ^a 0.0002	0.138 ± ^{ac} 0.0002	0.129 ± ^{ac} 0.0002
Stir-fried kenger (extracted in water)	0.405 ± 0.0002	0.390 ± ^c 0.0002	0.434 ± 0.0002	0.470 ± 0.0003	0.593 ± ^b 0.0004	1.025 ± ^b 0.0005
BHT Standard Sample	0.125 ± 0.0001	0.165 ± 0.0002	0.185 ± 0.0001	0.195 ± 0.0001	0.201 ± 0.0002	0.260 ± 0.0003

Analysis in each group (n = 5) was calculated as mean ± SD (standard deviation) and statistical differences between the cooked groups

BHT Butylated hydroxyl toluene

^a significant loss in antioxidant activity as compared to other groups (p < 0.05)

^b significant increase in antioxidant activity as compared to other groups (p < 0.05)

^c significant decrease in antioxidant activity as compared to its lower sample concentrations (p < 0.05)

Table 4 Antioxidant activity values of raw and cooked kenger samples with increasing concentration of sample: ABTS method (% TEAC ± SD)

Sample Concentration	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	150 mg/mL	300 mg/mL
Raw kenger	N.D	N.D	5.2 ± 0.75	11.3 ± 1.42	22.5 ± 1.53	52.5 ± 3.45
Boiled kenger (extracted in methanol)	5.4 ± 0.38 ^a	5.1 ± 0.45 ^a	36.5 ± 3.5	64.8 ± 8.9	79.5 ± 3.7	90.0 ± 5.55
Boiled kenger (extracted in water)	N.D	10.1 ± 5.2	18.2 ± 1.2	25.4 ± 2.4	40.2 ± 2.1	89.5 ± 5.52
Stir-fried kenger (extracted in methanol)	28.8 ± 2.25	55 ± 4.18	71.6 ± 8.25 ^b	88.8 ± 11.24 ^b	89.5 ± 6.75 ^b	89.8 ± 6.40
Stir-fried kenger (extracted in water)	7.6 ± 0.21	18.4 ± 0.75	27.2 ± 1.21	42.5 ± 1.61	80.6 ± 3.41	80.4 ± 3.5

Analysis in each group (n = 5) was calculated as mean ± SD (standard deviation) and statistical differences between the cooked samples groups

^a significant loss in antioxidant activity as compared to other groups (p < 0.05)

^b significant increase in antioxidant activity as compared to other groups (p < 0.05)

Table 5 Correlation between the antioxidant activity assessing methods

	DPPH 25 mg	DPPH 50 mg	DPPH 75 mg	DPPH 100 mg	DPPH 150 mg	DPPH 300 mg	SCALE
FRAP 25 mg	0.359						
ABTS 25 mg	-0.763						-1.00
FRAP 50 mg		0.100					-0.70
ABTS 50 mg		-0.700					-0.50
FRAP 75 mg			0.100				-0.30
ABTS 75 mg			* -0.900				-0.10
FRAP 100 mg				0.100			0.00
ABTS 100 mg				* -0.900			0.10
FRAP 150 mg					0.300		0.30
ABTS 150 mg					-0.400		0.50
FRAP 300 mg						-0.700	0.70
ABTS 300 mg						-0.100	1.00

*Correlation significant (rho = -0.90 p < 0.05)

Discussion

Phenolic compounds in raw and cooked kenger samples

In general, the concentration of phenolic compounds in plants has been reported to be influenced by genetic factors, environmental conditions (soil, sun, rain, climate), cultivation methods (organic greenhouse), harvest time, post-harvest processes, storage conditions and cooking practices (Kalkan & Yucesan, 2013) and variables in laboratory assessment methods. In a study, GC/MS methods were used to analyse the phytochemicals in kenger plants collected in March in Galil region of Israel. Thirty nine compounds including sterols, esters, phenolic compounds, saturated and unsaturated fats, acids and aromatic compounds were identified in the methanol and hexane extracts of the sample (Kadan et al., 2018). In another study conducted in Iran, the authors reported considerably large amounts of caffeic, chlorogenic and neo-chlorogenic acid derivatives in shoots and leaves of kenger plant locally harvested in the month of May (Haghi 2011).

In this study, vanillic acid ($18.755 \pm 0.606 \mu\text{g/g}$), fumaric acid ($16.211 \pm 0.524 \mu\text{g/g}$) and hydrobenzoic acid ($0.129 \pm 0.004 \mu\text{g/g}$) were detected among the 25 phenolic compounds studied in raw kenger. In addition caffeic acid was detected in boiled ($0.340 \pm 0.010 \mu\text{g/g}$) and stir-fried ($1.291 \pm 0.042 \mu\text{g/g}$) kenger. In a study conducted on kenger collected from the Upper Euphrates region of Turkiye (Elazig, Tunceli, Bingol, Diyarbakir) during Spring, major phenolic compounds determined were quercetin (0.5–22.5 mg/kg), naringenin (1.25–7.50 mg/kg), resveratrol (1.25–3.75 mg/kg), kaempferol (10.86–21.8 mg/kg) and myricetin (1.25–6.75 mg/kg) (Yildiz, 2014). These differences may have been due to the climatic conditions and agrochemical properties of the soil where the plant was grown. Fumaric acid decreased to a large extent on boiling and stir-frying, the loss was more in boiled samples. Vanillic acid on the other hand, decreased significantly after stir-frying. Caffeic acid was not detected in raw samples but was detected in boiled and stir-frying. Phenolics in food matrices are in bound as soluble conjugates or insoluble forms. Phenolic acids have been reported to occur mostly in the insoluble or bound forms in food. Processing techniques as thermal treatment, or enzymatic/acid/alkaline hydrolysis may release insoluble bound phenolics into more bioactive moieties (Acosta-Estrada et al., 2014; Moon & Shibamoto, 2010). Caffeic acid has also been suggested to be released from Chlorogenic acid bound complex, as a result of enzymatic hydrolysis (Acosta-Estrada et al., 2014). In this study, caffeic acid was detected after thermal treatment (Table 1) which may be due to conversion of its bound form into more bio-active forms.

On the other hand, thermal treatments as boiling and stir-frying have been reported to have decreased phenolic compounds in vegetables (Kalkan & Yucesan, 2013; Yao et al., 2011). Ribas-Agusti et al., suggested in their study that, boiling, frying and other cooking methods with direct contact with the cooking media have higher effect on the phenolic compounds content than methods with indirect contact, such as steaming, where leaching losses are more limited (Ribas-Agusti et al., 2018). On the other hand, some other studies have suggested that domestic frying procedures in vegetable oil may increase phenolic compounds and antioxidant activities due to the presence of antioxidant vitamins in oils and thermal stability of bio-active compounds (Gangcheng et al., 2019; Gomez-Alonso et al., 2003).

Vitamin C in raw and cooked kenger samples

On determining the vitamin C contents in raw and cooked kenger samples, significant ($p < 0.05$) loss in the vitamin was found in both boiled and stir-fried samples as compared to the raw ones (Table 2). Vitamin C content in boiled kenger decreased by 10.2% as compared to raw samples from $7.104 \pm 0.074 \mu\text{g/g}$ to $6.812 \pm 0.22 \mu\text{g/g}$. Fried kenger had an intermediate vitamin C value ($6.898 \pm 0.072 \mu\text{g/g}$). Vitamin C (ascorbic acid) is a thermo labile, water soluble vitamin and is expected to oxidize to dehydro-ascorbic acid when subjected to heat treatment. Loss in vitamin C content during thermal processing was due to its degradation to oxides and peroxides in presence of enzymes like ascorbate oxidase and peroxidase (Gomez et al., 2023; Zeng, 2013). However, the loss was lesser than expected and as reported to be as high as 60% by some studies (Idowu et al., 2018; Leskova et al., 2006). This has also been suggested by Akbiyik et al.'s study where the authors have reported the protection of nutritional ascorbic acid levels in foodstuffs in the presence of fruit acids as fumaric, citric, oxalic, tartaric, malic and malonic acids, by preventing metal-catalyzed oxidation reactions (Akbiyik et al., 2012). Favorable results regarding vitamin C retention in stir-fried kenger samples, in this study, may have resulted due to quick frying time (5 min). Furthermore, it has been reported that antioxidant vitamins (tocopherols) in vegetable oils may protect the thermo-oxidative stability of the food product (Gangcheng et al., 2019; Gomez-Alonso et al., 2003).

In a study conducted on broccoli by Yuan et al., (2009), all cooking processes except steaming caused significant loss in vitamin C content ($p < 0.05$) as compared to the raw vegetable. The maximum loss was detected in frying followed by boiling (38% and 33% respectively). Better retention of vitamin C retention was noted with micro-waving with a loss of approximately 16%. Steaming on

the other hand, did not cause any significant loss in vitamin C compared to the raw sample (Yuan et al., 2009). In another study (Zeng, 2013) conducted on spinach, broccoli and lettuce, vitamin C loss was recorded following steaming, microwaving and boiling of the vegetables. Minimum loss was recorded for steaming (approximately 11%) and maximum in boiling (up to 55%).

Antioxidant activities of raw and cooked kenger samples

It has been suggested that contents of antioxidants in plant products differ according to the plant species, the part of the plant analyzed, the harvest time, geographical region of the plant, analysis methods and reagents used (Kalkan & Yucesan, 2013). In this study, radical scavenging potentials (antioxidant activities) of the kenger extracts were analyzed at different concentrations (25 mg/mL, 50 mg/mL, 75 mg/mL, 100 mg/mL, 150 mg/mL and 300 mg/mL) of the sample by DPPH, FRAP and ABTS methods. In general, antioxidant activities of samples for all assays increased with rising concentration of the samples however, there were fluctuations in a few, which may have resulted from some unexplained interfering factors. Antioxidant activity of boiled samples decreased significantly ($p < 0.05$). However, antioxidant activity of stir-fried samples were close to raw ones as per FRAP and DPPH but increased as per ABTS, possibly due to chemical changes during high thermal treatment and antioxidants (Tocopherols) in vegetable oil.

In this study, maximum antioxidant activity (1.860 ± 0.0006 $\mu\text{mole/mg/mL}$) was observed in stir fried kenger samples extracted in methanol, at the maximum concentration of 300 mg/mL, in the FRAP method. This was followed by raw kenger (1.350 ± 0.0007 $\mu\text{mole/mg/mL}$). DPPH method exhibited similar results with fried kenger (extracted in water at a dose of 300 mg/mL) showing highest antioxidant activity (1.025 ± 0.0005 μg) followed by raw samples (0.731 ± 0.0008 μg). However, the antioxidant values obtained in the FRAP method was found to be higher than those obtained by DPPH method. In the ABTS method at the highest dose of 300 mg/mL, boiled kenger samples extracted in methanol and water exhibited high antioxidant activities upto 90% TEAC. However, at lower doses methanol extracts of fried kenger exhibited higher antioxidant activities as compared to others (Table 4). No correlation was found between these three methods with respect to antioxidant activities of the extracts at different concentration. This may have risen from variable factors in the laboratory and the number of samples being relatively less (triplicates for each extraction), the correlation obtained may not be considered reliable.

In a study conducted by Konak et al., three different methods were used to determine the antioxidant capacity

of the kenger plant. In terms of total antioxidant capacity, the CUPRAC method gave the highest and ABTS method, the lowest results. When evaluated in terms of extractable phenolic compounds, as per CUPRAC method the mean value was calculated to be 1493.99 $\mu\text{mol Trolox/100g}$, followed by DPPH with 916.05 $\mu\text{mol Trolox/100g}$ and ABTS with 367.01 $\mu\text{mol Trolox/100g}$ (Konak et al., 2017).

In the study by Jimenez et al., (2009) on the effect of cooking methods on the antioxidant activity (ABTS radical anion scavenging) of vegetables, boiling of garlic exhibited the highest losses in the antioxidant activity (over 50%) followed by boiled zucchini and celery being 30% and 14% respectively. Other cooking methods as frying, pressure cooking and baking increased antioxidant activities (indicated by increases in TEAC value) of green beans and carrots. On the other hand, sweet chard and cauliflower lost 30–40% of antioxidant activity on frying. In Kalkan and Yucesan's (2013) study however, antioxidant activity was found to decrease on cooking, maximum loss was observed on frying up to 61% followed by boiling in large amounts of water (Jimenez et al., 2009; Kalkan & Yucesan 2013).

Limitations of the study

The number of samples included in the study were relatively small due to budget issues and therefore the correlations obtained between parameters were not reliable. Furthermore, in future studies cooking time must be taken into consideration since losses in antioxidants may increase with cooking time. Needless to say, the antioxidant potential of food may not be reflected in the same pattern in human body, since metabolism of food may be affected by several factors including genotypes of individuals. In vitro and in vivo studies are required to fully understand the health potential of bioactive compounds in kenger.

Conclusion

Kenger is one of the endemic plants belonging to south east part of Turkiye having antioxidant properties and potential health benefits owing to the presence of bioactive compounds. Although, kenger is consumed by the locals in different forms, this study tried to elucidate the impact of common cooking methods (boiling in water and stir-frying in oil) on nutritional contents of kenger with respect to antioxidants as phenolics and vitamin C. Although cooking decreased antioxidants in kenger to a certain extent, thermal treatment sin oil increased certain phenolic compounds and antioxidant activities. Moreover, inclusion of condiments, herbs, olive oil, yogurt during service would not only increase palatability but also add to the health benefits.

As future strategies for kenger in Türkiye, proper marketing strategies must be adapted so that not only local consumers but consumers all over the country can avail the product through supermarket chains. In addition, awareness of local farmers must be raised in this context since self-grown kenger plant is facing the risk of extinction due to excessive use of pesticides and poor agricultural practices.

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Authors' contributions

Kalkan I. took major role in data collection, analysis and writing of the paper. Eyupoglu O.E. was involved in data analysis and evaluation. Karatas S. was involved in writing of the paper. Aissaoui Z. E.M. and Anik R. was involved in data collection.

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Data availability

Data related to this study can be shared upon request.

Declarations

Ethics approval and consent to participate

No approval was sought since the study involved research work on plant material.

Consent for publication

The authors provide their consent for publication of this manuscript and research data.

Competing interest

Authors declare no conflict of interest regarding research or publication of this paper.

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