



Impact of Aqueous Extracts of Turkish Wild Edible Plants on Acrylamide Formation in Potato Crisps

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HIGHLIGHTS

- A Turkish wild edible plant named ribwort plantain had the potential of decreasing acrylamide content of potato crisps.
- p-Coumaric acid, chlorogenic acid, and p-hydroxy benzoic acid may have played a role in this acrylamide reduction.
- There was no significant correlation between physicochemical properties of plant extracts and acrylamide level of crisps.

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Acronyms and abbreviations

ABTS=2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
CUPRAC=Cupric ion Reducing Antioxidant Capacity
DPPH=1,1-Diphenyl-2-Picrylhydrazil
FRAP=Ferric Reducing Antioxidant Power
LC-MS/MS=Liquid Chromatography-Tandem Mass Spectrometry
LOD=Limit of Detection
LOQ=Limit of Quantification
TAC=Total Antioxidant Capacity
TFC=Total Flavonoid Content
TPC=Total Phenolic Content

ABSTRACT

Background: Antioxidants have the ability to influence acrylamide formation. This study aimed to evaluate the impact of aqueous extracts of six wild edible plants on the acrylamide formation in potato crisps.

Methods: Sliced potatoes were submerged in the plant extracts at a concentration of 0, 5, and 10 g/L for 1, 5, and 10 min. Before being fried and their acrylamide levels were calculated by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

Results: Aqueous extract of ribwort plantain was found the most effective trial at 10 g/L for 5 min because it reduced acrylamide concentration by 57% compared to control without significantly affecting potato crisps' sensory and color parameters ($p>0.05$). The aqueous extract of shepherd's-needle yielded the highest Total Antioxidant Capacity (TAC) in 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; ABTS) and Cupric ion Reducing Antioxidant Capacity (CUPRAC) assay, the highest Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). Similarly, no significant correlation was found between TAC, TPC, and TFC of watery plant extracts with acrylamide level of potato crisps produced after immersion of these extracts (at 5 g/L for 5 min).

Conclusion: Wild edible plants have the potential to be used for acrylamide reduction in potato crisps.

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Introduction

When a food is exposed to high-temperature during processing, a probable human carcinogen acrylamide can

form (EFSA CONTAM, 2015; Gökmən and Palazoğlu, 2008; Morales et al., 2014). The main pathway in the

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formation of acrylamide is Maillard reaction between reducing sugar and amino acids (Wang and Xu, 2014). The amount of acrylamide may vary importantly in food products, depending on different parameters such as the composition of food material, the process of preparation, process temperature, or processing time (Stadler and Scholz, 2004; Stojanovska and Tomovska, 2015). Previous studies showed that the main dietary contributors to acrylamide are French fries, potato crisps, crackers, breakfast cereals, cookies, and coffee products (Akgün and Arıcı, 2019; EFSA CONTAM, 2015; NIH, 2017). According to EFSA CONTAM (2015), dietary exposure levels to acrylamide showed a health concern and the acrylamide amount in foods has not continually diminished recently. European Commission (EC, 2017) established mitigation measures and benchmark levels for the acrylamide reduction in food and new European Union (EU) acrylamide legislation became valid in April 2018 (Bonwick and Birch, 2019). It was thought that this situation will direct food business operators to take into account the benchmark levels for acrylamide defined by the regulation and to implement mitigation measures for decreasing the acrylamide level in their food products. However, there are still notifications in the Rapid Alert System for Food and Feed (RASFF) portal related to foods (especially biscuits and potato crisps) exceeding benchmark levels for acrylamide.

Most of the study on this aspect aimed to decrease acrylamide level in fried potato products because these products are widely consumed by consumers worldwide (Morales et al., 2014) and they are at the forefront of contributing to dietary acrylamide intake (Wilson et al., 2006). Previously, modification in raw materials' properties and/or technological processes were used for reduction in acrylamide amount of potato crisps and French fries (Liyanage et al., 2021; Tajner-Czopek et al., 2021). Using potato variety having lower reducing sugars and asparagine levels may aid acrylamide concentration mitigation in the final product (De Wilde et al., 2005). The use of thicker potato slices, blanching potato slices in an acidic solution, the use of various plant extracts, and the addition of phenolic acids, soaking potato slices in a solution containing proanthocyanidins and polyphenols, the use of certain oil type for frying, the use of enzyme asparaginase, the addition of some amino acids (e.g. glycine and glutamine), the use of vacuum fryer, the lower the process temperature, and the shorter process time are just some strategies for acrylamide reduction in fried potato products (Pedreschi et al., 2008; Tajner-Czopek et al., 2021). However, many of these techniques may have negative effects on color, texture, and taste of foods (Morales et al., 2014). Therefore, it is very crucial to find methods for decreasing acrylamide level in foods without adversely impacting their quality attributes.

People's interest in wild plants has been increasing in recent years. The beneficial effects on health of these plants have made their uses for different purposes more attractive (Schunko et al., 2015). Plant extracts possess many phenolic compounds like flavonoids, phenolic acids, coumarins, lignans, and tannins (Kähkönen et al., 1999; Manzocco et al., 1998). Anatolian peninsula is in a very advantageous position in terms of natural resources due to its biogeographical location and it hosts many wild plants. For this reason, wild edible plants have an important place in Turkish cuisine, especially in the Aegean and Mediterranean regions (Karaca et al., 2015; Kök et al., 2020). The most commonly used herbs in Aegean cuisine are tangle, blessed thistle, sorrel, mallow, sea beans, asparagus, peppergrass, and rocket (Kök et al., 2020). Past studies showed that some wild edible plants from different regions of Türkiye can exhibit high antioxidant activity (Özen, 2010; Sarikurkcu et al., 2016; Taskin and Bitis, 2016). Natural antioxidants from plant materials may eliminate the negative impact of acrylamide by reducing free radical levels (Ibrahim et al., 2019). However, some wild plants could be poisonous (Cornara et al., 2018) so non-poisonous species must be used for this purpose. Republic of Türkiye Ministry of Agriculture and Forestry (2022) published a plant list (continuing to be developed) depending on whether plants are allowed to be used for food sources. According to this list, the positive classification of a plant means that the parts specified in the list are allowed to be used as food sources and negative classification means that their uses as food sources are not allowed in Türkiye.

To our knowledge, this is the first study for the evaluation of the effect of wild edible plants on the acrylamide formation in potato crisps. The aim of this study was to evaluate the success of aqueous extracts of six various plants on acrylamide mitigation in potato crisps by considering sensory and color parameters and to determine phenolic compounds profile of extracts in order to assess the reason behind acrylamide level change.

Materials and methods

Chemicals and consumables

Acrylamide (99.3%) was purchased from Dr. Ehrenstorfer GmbH (Germany). Oasis HLB and Bond-Elut Accucat SPE cartridges were from Waters (USA) and Varian (USA), respectively. 1,1-Diphenyl-2-Picrilhydrazil (DPPH), copper (II) chloride ($CuCl_2$), 2,9-Dimethyl-1,10-phenanthroline (Neocuproine), trolox ((\pm)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), potassium persulfate ($K_2S_2O_8$), gallic acid, aluminium chloride ($AlCl_3$), quercetin, 2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate trihydrate

(C₂H₃NaO₂.3H₂O), methyl formate (C₂H₄O₂), glacial acetic acid, ferric chloride hexahydrate (FeCl₃.6H₂O), and acrylamide-d3 (AA₃) were acquired from Sigma-Aldrich (Steinheim, Germany). Zinc sulfate heptahydrate (ZnSO₄.7H₂O), Potassium hexacyanoferrate (II) trihydrate, ammonium acetate (C₂H₇NO₂), 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (>98%), Folin-Ciocalteu's phenol reagent, sodium hydroxide, sodium carbonate (Na₂CO₃), Copper II Sulphate (CuSO₄), sodium potassium tartrate (NaKC₄H₄O₆), formic acid (98%), methanol, ethanol, acetonitrile, and water were from Merck (Darmstadt, Germany). The phenolic compound standards were purchased from Extrasynthese (Genay Cedex, France) and Sigma-Aldrich Chemie (USA). Asahipak NH2P-50G 4E (250x4.6mm) column was from Shodex (USA). Inertsil ODS-4V (4.6×150 mm; 3 µm) LC column was from GL Sciences (Japan). C18 (4×3mm) guard column was from Phenomenex (Torrance, CA). Palm olein oil without any added natural or synthetic antioxidants was obtained from Upfield Istanbul Gida A.S.

Plants and potato materials

Wild edible plants stated in Table 1 were collected from the Aegean Region of Türkiye (Urla, Izmir) in April 2020. All used plants except shepherd's-needle were classified as positive in the plant list published by Republic of Türkiye Ministry of Agriculture and Forestry (2022). No information was given in the list about shepherd's-needle but it has been consumed for a long time in Europe as a vegetable. The coordinates of where we gather the plants were in Barbaros village and antic Klozemania ruins (38°18'19.8"N 26°36'30.2"E and 38°21'44.2"N 26°46'00.8"E). After picking, fresh plants were cleaned manually and dried in an oven (Dedeoğlu, Türkiye) at 40 °C for 24-30 h. A laboratory mill (FRITSCH GmbH, Germany) was used to powder dried plants and they were kept at -18 °C until used.

Potato (*Solanum tuberosum* L.) sample variety Agria was bought from the retail market in Bursa, Türkiye. Potatoes were washed, peeled, and sliced by using a horizontal slicer (Seles M 250, Italy) to get uniform and thin slices (thickness -1.5±0.1 mm). Also, a digital caliper (Mitutoyo Model-150X, Japan) was utilized to control thickness. Then, a plastic cutting mold was utilized to get circular slices (diameter: 36 mm).

Preparation of plant extracts and potato crisps

The extract preparation method was designed by considering previous studies done by Albu et al. (2004), Jiang et al. (2011), and Kamiloglu et al. (2014). Every dried and powdered plants (2.5 g) was extracted with 50 ml water in a 35 kHz ultrasonic bath (Bahdelin Sonorex,

Germany) at 50-55 °C for 30 min. The treated samples were centrifuged (Eppendorf 5,804, Germany) for 10 min at 4,000 rpm and the supernatant was taken. The extraction procedure was repeated and other 50 ml of water was added to the pellet. Two supernatants were united and 100 ml final volume was obtained. This final volume was filtered by using filter paper (1,535, Filter-Lab). Plant extracts at 5 g/L and 10 g/L concentrations were prepared by diluting obtained extracts with distilled water. These extracts were used on the same day. The effect of extraction solvent on Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) was also evaluated. For that purpose, 80% methanol in water and 80% ethanol in water were prepared and used as solvents during the extraction of dried plants in addition to water. For potato crisps preparation, sliced potatoes were firstly immersed in water or different watery plant extracts for varying times (1, 5, and 10 min) at 24±1 °C; and then slices were put on paper towel and dried in an oven at 100 °C for 10 min. Afterwards, a deep fryer (Arçelik Arz 40 FZ, Türkiye) filled with palm olein oil was used for frying potato slices at 170±2 °C for 110 s. The oil temperature was controlled using a thermometer and oil was changed after every third uses.

Physical, chemical, and antioxidant properties

-TPC determination

All spectrophotometric measurements were done in UV-1280 UV-Vis spectrophotometer (Shimadzu, Japan). While determining TPC of extracts, the method offered by Apak et al. (2008) was used. A calibration curve was prepared using gallic acid solution. The results were expressed as mg Gallic Acid Equivalents (GAE)/g sample.

-TFC determination

TFC was measured spectrophotometrically as described by Brighente et al. (2007) and Bouyahya et al. (2018). A calibration curve for quercetin was used for the calculation of TFC. The results were expressed as the quercetin equivalents (QE)/g sample.

-TAC determination

TAC was measured by four different assays (DPPH, Ferric Reducing Antioxidant Power (FRAP), Cupric ion Reducing Antioxidant Capacity (CUPRAC), and ABTS. These determinations were performed in triplicate.

-DPPH method

DPPH method was performed using the method described by Singh et al. (2002). Inhibition values (%)

and the corresponding 0.0025-0.01875 mM values of the trolox solution were used to obtain the standard calibration graph. The calibration graph was used to calculate antioxidant capacities of extracts and results were stated as $\mu\text{mol trolox Equivalent (TE)}/\text{sample}$.

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}}$$

Where $\text{Abs}_{\text{blank}}$ is the absorbance of the control and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample (Bouyahya et al., 2018).

-CUPRAC method

The CUPRAC assay developed by Apak et al. (2008) was used. Antioxidant capacity calculation was carried out using a standard curve prepared with trolox solution and results were expressed as $\mu\text{mol TE/g sample}$.

-ABTS method

Antioxidant capacity analysis of extracts by ABTS assay were done according to the method described by Apak et al. (2008). Trolox was used as a standard in the determination of antioxidant capacity. The results were given as $\mu\text{mol TE/g sample}$.

-FRAP method

The method offered by Thaipong et al. (2006) was used. The results were given as $\mu\text{mol TE/g sample}$.

-Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS)

Phenolic compound analysis was performed using an Agilent 6,550 LC-ESI-Q-TOF-MS (Agilent Technologies, USA) system equipped with a Poroshell 120 EC. The instrument conditions were set as follows: drying N_2 gas flow rate, 14 L/min; temperature, 175 °C, nebulizer, 45 psig; capillary voltage of 3,500 V; fragmentor 300; skimmer, 65 V. The sample injection volume was 5 μl and flow rate was 0.6 ml/min. Formic acid (0.1%, v/v) in water (A) and formic acid (0.1%, v/v) in acetonitrile (B) were used as mobile phases. The applied gradient was as follows: 2 min, 5% B; 2-20 min, 5-60% B; 20-22 min, 60-95% B; 22-28 min, 95% B; 28-32 min, 5% B. C18 analytical column (4.6x100 mm, 2.7 μm) and the negative ionization mode were used. External calibration curves were prepared with the phenolic standards at 5 different concentration levels from 25-400 $\mu\text{g/L}$ (Karaagac and Şahan, 2020).

-Quantification of pH in powdered plants

AOAC 943.02 method (Araujo et al., 2016) was used to measure the pH value of powdered plants. The pH

measurements were performed using a pH meter (Mettler-Toledo, Switzerland).

-Quantification of sugars in powdered plants

AOAC Official Method 979.23 was followed for total reducing sugar calculation (AOAC, 1979). Shimadzu RID-10a High Performance Liquid Chromatography (HPLC) system and Shodex Asahipak NH2P-50G 4E (4.6 mm I.D.x250 mm) column were used for total reducing sugar (glucose+fructose) calculation (%). The mixture of acetonitrile and water (75:25, v/v) was prepared as a mobile phase. Injection volume, flow rate, and column oven temperature were 10 μl , 1 ml/min and 30 °C, respectively.

Method validation and acrylamide analysis in crisps

For the linearity study, a matrix-matched calibration curve was constructed at six concentrations of acrylamide (30, 60, 120, 240, 480, and 960 $\mu\text{g/kg}$) by using one concentration of internal standard (500 $\mu\text{g/kg}$). One g of raw potato was spiked with 200 μl of each acrylamide standard and 100 μl internal standard to establish this curve. Linear regression analysis (relative response versus relative concentration) was performed to compute the coefficient of determination (R^2).

For calculation of Limit of Detection (LOD) and Limit of Quantification (LOQ), blank samples (without detectable level of target analyte) were spiked with acrylamide at 30 $\mu\text{g/kg}$ level and 10 independent studies were conducted. Standard deviation of results was calculated according to equation 1. LOD and LOQ are equal to 3 and 10 times of standard deviation, respectively (Republic of Türkiye Ministry of Agriculture and Forestry, 2018a).

$$\text{SD} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{N-1}} \quad (\text{Eq 1})$$

where SD: the sample standard deviation; x_i : each value in the data set; \bar{x} : mean of all values in the data set; N: number of values in the data set

Repeatability and reproducibility Relative Standard Deviation were expressed as RSD_r% and RSD_R%, respectively. For repeatability study, samples were prepared by each analyst according to the sample preparation procedure and injected into High-performance liquid chromatography (LC-MS/MS) at 2 different concentrations and 6 parallel repeats. For the reproducibility study, samples were extracted and injected into the instrument at 2 different concentrations on 3 different days. Within the scope of repeatability and reproducibility studies, 30 and 240 $\mu\text{g/kg}$ concentrations were used. Conformity assessment of RSD% values were done according to guide for method validation/verification in chemical and physical analysis (Republic of Türkiye Ministry of Agriculture and Forestry, 2018a).

Expanded relative combined uncertainty was determined by multiplying the relative combined uncertainty (equation 2) with a coverage factor $k=2$ at the confidence level of 95% m(Republic of Türkiye Ministry of Agriculture and Forestry, 2018b).

$$U(X) = \sqrt{\frac{U(c_0)}{c_0} + \frac{U(RSDr)^2}{RSDr} + \frac{U(RSDR)^2}{RSDR} + \frac{U(GK)^2}{GK}} \quad (\text{Eq 2})$$

$U(X)$: the relative combined uncertainty; $U(c_0)$: measurement uncertainty for calibration curve; c_0 : the determined solution concentration; $U(RSDr)$: measurement uncertainty for repeatability; $U(RSDR)$: measurement uncertainty for reproducibility; $U(GK)$: the measurement uncertainty for recovery

Acrylamide extraction was done according to the method proposed by Roach et al. (2003). One g ground sample was weighed into a 50 ml centrifuge tube and AAd3 solution was added at 500 $\mu\text{g}/\text{kg}$ level (100 μl from 5 ppm AAd3 solution). After 5 ml hexane addition, the tube was mixed. Subsequently, 9.9 ml of distilled water were added to the tube and it was shaken for 20 min on a multi-reatox tube shaker (Heidolph Schwabach, Germany). The tube was centrifuged at 9,000 rpm for 15 min. Five ml of aqueous supernatant was separated and filtered using a PVDF syringe filter (0.45 μm). Then, 1.5 ml of filtered extract was added to preconditioned Oasis HLB cartridges (conditioned with 3.5 ml methanol (MeOH) and 3.5 ml water). The extract passed through the sorbent was removed and 0.5 ml water was used to wash the cartridge. Later, 1.5 ml of water was loaded onto the cartridge and the eluant was collected. Preconditioned a Bond Elut Accucat SPE cartridge (conditioned with 2.5 ml MeOH and 2.5 ml water) was loaded with the obtained extract. The first 0.5 ml of the eluate was removed and the remaining part was taken into a vial. All samples were injected to Shimadzu LC-MS/MS 8040 with an ESI source operating in positive ionization mode. Operating conditions were given in the article by Akgün et al. (2021). For quantification, a calibration curve for relative concentration vs relative response was developed. The amount of acrylamide was calculated by considering internal standard. Five hundred ng of internal standard was added to a 1 g portion, so acrylamide ($\mu\text{g}/\text{kg}$) = $((500 \text{ ng}) \times (\text{area } m/z 55)) / ((\text{area } m/z 58) \times (\text{g portion}) \times (\text{response factor}))$.

Sensory and color evaluation of potato crisps

Sensory evaluation was performed on potato crisp samples; potato crisps containing lowest acrylamide level (immersed in watery ribwort plantain extract at 10 g/L for 5 min) and control potato crisps (immersed in distilled water at 0 g/L for 5 min) in order to evaluate the impact of plant extract on sensory attributes, including

texture, color, appearance, taste, odour, and overall acceptability. The samples were rated on a 10-point scale. The samples placed randomly were served to each panelist (n=9) (Basuny et al., 2009; Carpenter et al., 2000). The color of these potato crisps (E and C) was determined by Hunter-Lab (USA). Results were given as L*, a*, and b*.

Statistical analysis

SPSS 21 (IBM SPSS Statistics 21) package programme was used to process obtained results. ANOVA, Duncan's multiple range test, T-test or pearson's correlation test were utilized for the statistical analyses of some results.

Results

Determination of TAC, TPC, and TFC of the extracts

Four different methods (DPPH, ABTS, FRAP, and CUPRAC) were used to measure TAC values of plant extracts as shown in Table 2. Statistically significant differences were observed among TAC, TFC, and TPC values of six plants prepared with different solvents ($p<0.05$). When each plant's extract was evaluated separately, TAC values of methanolic and ethanolic extracts of plants were commonly found similar statistically. On the other hand, TAC of watery extract of plants were found lower than methanolic and ethanolic extracts. Similarly, TPC and TFC of methanolic and ethanolic extract measured were generally higher than TPC and TFC of watery extract. Among watery extracts, the water extract of shepherd's-needle showed the highest TAC ($117.5 \pm 4.50 \mu\text{mol TE/g sample}$) based on CUPRAC method and the water extract of sow-thistle had the lowest TAC value ($5.15 \pm 2.30 \mu\text{mol TE/g sample}$) based on DPPH method. Among ethanolic and methanolic extracts, ethanolic extract of sow-thistle had the highest TAC ($189.00 \pm 8.00 \mu\text{mol TE/g sample}$) according to CUPRAC method.

Method validation of acrylamide

Over the range of 30 and 960 $\mu\text{g}/\text{kg}$, the calibration curve had a good value of coefficient of determination ($R^2 \geq 0.999$). LOD and LOQ values were found as 4.38 ve 14.59 $\mu\text{g}/\text{kg}$, respectively. LC-MS/MS chromatogram of acrylamide and AAd3 fragment ions were shown in Figure 1.

Determination of acrylamide levels in potato crisps

Table 3 showed acrylamide concentrations of potato crisps produced from sliced potatoes treated with various plant extracts or distilled water. The acrylamide level of

potato crisps was found between $164.66 \pm 31.29 \text{ } \mu\text{g/kg}$ and $1,390.67 \pm 264.23 \text{ } \mu\text{g/kg}$. Statistically significant differences were observed among produced potato crisp samples in terms of acrylamide levels ($p < 0.05$). Contradictory results were obtained because some applications increased acrylamide level of potato crisps but others decreased. The lowest acrylamide level ($164.66 \pm 31.29 \text{ } \mu\text{g/kg}$) was obtained in the potato crisps made from potatoes immersed in ribwort plantain extract for 10 g/L for 5 min. Compared to control (0 g/L, 5 min), reduction percentage of 57% was achieved. It was seen that when raw potatoes were soaked with watery shepherd's-needle, milk thistle, garden sorrel, and sow-thistle plant extract, acrylamide levels in fried potatoes generally could not be decreased importantly with increased concentration and time (Table 3). As a general, soaking sliced potatoes in water for 10 min led to a decrease in acrylamide reduction compared to 5 min.

Determination of reducing sugar and pH of plants

As seen in Table 4, there were significant difference in total reducing sugar and pH value ($p < 0.05$). Total reducing sugar level of ribwort plantain ($3.90 \pm 0.15\%$) was only significantly different from others.

Determination of phenolic acid content

In analysed watery plant extracts, phenolic acids (3-Hydroxybenzoic acid, gentisic acid, gallic acid, ellagic acid, quercetin-3-D-xyloside, protocatechuic acid, p-hydroxy benzoic acid (4-Hydroxy benzoic acid), chlorogenic acid, caffeic acid, ferulic acid, salicylic acid, o-Coumaric acid and p-Coumaric acid), flavonoids (isorhamnetin-3-O-glucoside, rutin hydrate (quercetin-3-O-rutinoside hydrate), isorhamnetin 3-O-rutinoside, syringetin-3-glucoside, epigallocatechin gallate, catechin, myricitrin (myricetin-3-O-rhamnoside), epicatechin, kaempferol, epicatechin gallate, quercentin (quercentin-3-

O-rhamnoside), and isorhamnetin) and stilbene (trans-resveratrol) were detected (Table 5). Although the total amount of phenolic compounds detected was the highest in garden sorrel extract, the largest acrylamide reduction was obtained when the aqueous ribwort plantain extract was used (10 g/L for 5 min). The chromatograms of p-Coumaric acid for external calibration at 400 ppb and ribwort plantain extract were shown in Figure 2.

Correlations between spectrophotometric assays and acrylamide level

For investigating correlations between spectrophotometric assays and acrylamide level, DPPH, ABTS, FRAP, CUPRAC, TPC, and TFC results of watery extracts of 6 plants and acrylamide results of potato crisps (at 5 g/L for 5 min) were used. According to Table 6, the highest correlation was obtained between TAC (ABTS assay) and TPC of the studied plant samples ($r=0.801$, $p < 0.01$). Significant correlations were found between the FRAP and CUPRAC assays, ABTS, and CUPRAC assays, DPPH and FRAP assays ($p < 0.01$ or $p < 0.05$). Also, it was found that acrylamide level was not in correlation with DPPH, ABTS, FRAP, CUPRAC, TPC, and TFC results (Table 6).

Determination of sensory properties and color of crisps

No significant difference ($p > 0.05$) was determined for the texture, color, appearance, taste, odour, and overall acceptability between potato crisps immersed in watery ribwort plantain extract at 10 g/L for 5 min (E) and potato crisps immersed in distilled water at 0 g/L for 5 min (C) (Figure 3). No statistically significant differences ($p > 0.05$) were found between color parameters (L^* , a^* , and b^*) of these potato crisps. Color values were L^* : 54.12 ± 0.30 , a^* : 1.95 ± 0.20 , b^* : 24.16 ± 0.18 for (C) and L^* : 53.55 ± 0.45 , a^* : 2.10 ± 0.15 , b^* : 23.75 ± 0.25 for (E).

Table 1: Name of wild plants from Aegean region of Türkiye used in the study

Family name	Botanical name	Plant name	Turkish name	Local names	Parts used
Polygonaceae	<i>Rumex acetosa</i> L.	Garden sorrel	Kuzukulagi	Labada, Efelek, Evelik	Leaves
Asteraceae	<i>Sonchus oleraceus</i> L.	Sow-thistle	Eşekgevregi/Eşekmarulu	Türkmen düdügü, Eşek helvası	Leaves
Papaveraceae	<i>Papaver rhoeas</i> L.	Common poppy	Gelinçik	Gelin ali, Gelineli, Lale kapırcığı	Leaves
Plantaginaceae	<i>Plantago lanceolata</i> L.	Ribwort Plantain	Sinirliot/ Damarotu	Dede kaşığı	Leaves
Asteraceae	<i>Silybum marianum</i> (L.) Gaertn.	Milk thistle	Meryem ana diken/ Devedikeni	Dayva diken	Leaves
Apiaceae	<i>Scandix pecten-veneris</i> L.	Shepherd's-needle	Çoban tarağı	İğnelik, Çoban iğnesi, Venüs tarağı	Leaves

Table 2: Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of watery, methanolic, and ethanolic extracts of 6 selected plants

Plant	Sample	DPPH ($\mu\text{mol TE/g}$)	ABTS ($\mu\text{mol TE/g}$)	FRAP ($\mu\text{mol TE/g}$)	CUPRAC ($\mu\text{mol TE/g}$)	TPC (mg GAE/g)	TFC (mg QE/g)
Garden sorrel	Watery extract	18.68 \pm 3.10 ^{cd}	51.26 \pm 7.60 ^b	26.40 \pm 3.90 ^d	62.50 \pm 2.50 ^b	29.25 \pm 3.10 ^d	5.10 \pm 0.50 ^b
	Methanolic extract	29.15 \pm 2.75 ^c	64.26 \pm 5.70 ^a	51.40 \pm 5.50 ^b	150.5 \pm 6.5 ^c	39.69 \pm 4.00 ^c	9.63 \pm 1.12 ^{de}
	Ethanolic extract	29.08 \pm 1.35 ^c	62.06 \pm 5.45 ^a	51.50 \pm 4.95 ^{ab}	153.5 \pm 6.35 ^c	40.63 \pm 3.50 ^{de}	13.38 \pm 1.34 ^{cd}
Sow-thistle	Watery extract	5.15 \pm 2.30 ⁱ	30.13 \pm 3.55 ^s	24.80 \pm 6.50 ^d	64.00 \pm 3.00 ^b	29.20 \pm 3.20 ⁱ	9.4 \pm 1.00 ^f
	Methanolic extract	29.65 \pm 8.90 ^c	58.60 \pm 5.20 ^{ab}	54.90 \pm 8.70 ^{ab}	180.00 \pm 7.00 ^{abc}	61.40 \pm 5.25 ^b	13.01 \pm 1.62 ^{de}
	Ethanolic extract	29.01 \pm 7.60 ^f	54.73 \pm 6.25 ^{ab}	54.00 \pm 8.10 ^{ab}	189.00 \pm 8.00 ^a	40.40 \pm 4.50 ^{de}	15.03 \pm 3.36 ^b
Common poppy	Watery extract	64.30 \pm 5.60 ^b	50.72 \pm 4.25 ^b	47.50 \pm 7.20 ^{bc}	110.0 \pm 5.00 ^d	39.06 \pm 2.50 ^c	8.60 \pm 1.00 ^{gh}
	Methanolic extract	84.80 \pm 8.90 ^a	64.25 \pm 7.50 ^b	52.90 \pm 4.10 ^{ab}	169.0 \pm 7.00 ^{cd}	44.78 \pm 4.25 ^{cd}	11.52 \pm 1.02 ^{def}
	Ethanolic extract	86.00 \pm 9.30 ^a	64.46 \pm 5.50 ^b	54.10 \pm 5.50 ^b	170.0 \pm 8.00 ^d	37.71 \pm 1.50 ^e	14.6 \pm 2.04 ^{bc}
Ribwort plantain	Watery extract	11.58 \pm 4.60 ^{ef}	37.00 \pm 4.50 ^f	24.60 \pm 3.90 ^d	82.5 \pm 3.50 ^g	30.25 \pm 2.00 ⁱ	7.20 \pm 0.30 ^{ghi}
	Methanolic extract	29.68 \pm 8.40 ^f	64.79 \pm 5.25 ^b	54.4 \pm 5.90 ^{ab}	179.5 \pm 7.50 ^{abc}	61.10 \pm 5.50 ^b	10.03 \pm 0.92 ^{fg}
	Ethanolic extract	29.36 \pm 7.10 ^f	64.05 \pm 6.50 ^b	53.1 \pm 4.70 ^{ab}	177.5 \pm 6.50 ^{bc}	49.81 \pm 4.30 ^c	9.58 \pm 1.72 ^f
Milk thistle	Watery extract	9.94 \pm 3.60 ^{ef}	36.53 \pm 3.20 ^f	44.9 \pm 3.40 ^b	86.5 \pm 3.50 ^g	24.55 \pm 3.40 ^j	5.72 \pm 0.87 ^{hi}
	Methanolic extract	28.47 \pm 6.60 ^f	63.39 \pm 7.60 ^a	50.9 \pm 5.50 ^{ab}	167.5 \pm 6.40 ^d	26.83 \pm 2.60 ⁱ	6.91 \pm 0.75 ^{ghi}
	Ethanolic extract	27.80 \pm 5.90 ^f	61.85 \pm 6.40 ^a	53.1 \pm 4.70 ^{ab}	173.0 \pm 3.50 ^{cd}	28.16 \pm 2.50 ⁱ	8.45 \pm 1.05 ^{gh}
Shepherd's-needle	Watery extract	15.92 \pm 3.60 ^{ef}	60.66 \pm 5.40 ^{ab}	37.8 \pm 3.70 ^f	117.5 \pm 4.50 ^d	46.75 \pm 3.70 ^d	10.69 \pm 1.45 ^{ef}
	Methanolic extract	26.17 \pm 4.50 ^{cd}	64.26 \pm 3.10 ^a	56.2 \pm 5.90 ^a	185.5 \pm 6.50 ^{ab}	72.31 \pm 4.20 ^a	25.62 \pm 3.07 ^a
	Ethanolic extract	26.74 \pm 6.10 ^{cd}	64.79 \pm 7.40 ^a	54.3 \pm 7.60 ^{ab}	179.5 \pm 7.50 ^{abc}	59.92 \pm 5.50 ^b	24.99 \pm 4.05 ^a

Results were represented as mean \pm SD (n=3). In the same column, different labels indicated a significant difference at $p<0.05$. The results of the different extracts were evaluated by Duncan's multiple range test.

DPPH=1,1-Diphenyl-2-picrilhydrazil; CUPRAC=Cupric ion reducing antioxidant capacity; ABTS=2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP=Ferric Reducing Antioxidant Power; TE=Trolox equivalent; GAE=Gallic Acid Equivalents; QE=Quercetin Equivalents

Table 3: The amount of acrylamide in produced potato crisps

Application	Application condition	Acrylamide level ($\mu\text{g/kg}$)
Soaking in distilled water (Control)	0 g/L, 1 min	687.17 \pm 130.56 ^{defg}
	0 g/L, 5 min	379.35 \pm 72.08 ^{ijklmo}
	0 g/L, 10 min	506.75 \pm 96.28 ^{fghijk}
Soaking in aqueous milk thistle extract	5 g/L, 1 min	516.37 \pm 98.11 ^{ghij}
	5 g/L, 5 min	238.41 \pm 45.30 ^{mo}
	5 g/L, 10 min	471.31 \pm 89.55 ^{ghijkl}
	10 g/L, 1 min	832.89 \pm 158.25 ^{cde}
	10 g/L, 5 min	703.58 \pm 133.68 ^{def}
	10 g/L, 10 min	824.22 \pm 156.60 ^{cde}
Soaking in aqueous garden sorrel extract	5 g/L, 1 min	636.38 \pm 120.91 ^{ghij}
	5 g/L, 5 min	423.94 \pm 80.55 ^{hijklm}
	5 g/L, 10 min	388.96 \pm 73.90 ^{ijklm}
	10 g/L, 1 min	1174.29 \pm 223.12 ^b
	10 g/L, 5 min	429.15 \pm 81.54 ^{ghijklmn}
	10 g/L, 10 min	651.74 \pm 123.83 ^{defgh}
Soaking in aqueous shepherd's-needle extract	5 g/L, 1 min	452.74 \pm 86.02 ^{ghijklm}
	5 g/L, 5 min	446.89 \pm 84.91 ^{ghijklmn}
	5 g/L, 10 min	465.04 \pm 88.36 ^{ghijklm}
	10 g/L, 1 min	1390.67 \pm 264.23 ^a
	10 g/L, 5 min	860.30 \pm 163.46 ^{cd}
	10 g/L, 10 min	1017.00 \pm 193.23 ^{bc}
Soaking in aqueous sow-thistle extract	5 g/L, 1 min	479.00 \pm 91.01 ^{ghijkl}
	5 g/L, 5 min	454.25 \pm 86.31 ^{ghijklm}
	5 g/L, 10 min	342.53 \pm 65.08 ^{ijklmo}
	10 g/L, 1 min	547.28 \pm 103.98 ^{fghij}
	10 g/L, 5 min	416.81 \pm 79.19 ^{hijklm}
	10 g/L, 10 min	1004.89 \pm 190.93 ^{bc}
Soaking in aqueous ribwort plantain extract	5 g/L, 1 min	284.63 \pm 54.08 ^{ijklmo}
	5 g/L, 5 min	277.85 \pm 52.79 ^{ijklmo}
	5 g/L, 10 min	365.70 \pm 69.48 ^{ijklmo}
	10 g/L, 1 min	465.98 \pm 88.54 ^{ghijklm}
	10 g/L, 5 min	164.66 \pm 31.29 ^o
	10 g/L, 10 min	224.27 \pm 42.61 ^{mo}
Soaking in aqueous common poppy extract	5 g/L, 1 min	516.00 \pm 98.04 ^{ghij}
	5 g/L, 5 min	401.50 \pm 76.29 ^{ijklm}
	5 g/L, 10 min	319.49 \pm 60.70 ^{ijklmo}
	10 g/L, 1 min	687.46 \pm 130.62 ^{defg}
	10 g/L, 5 min	351.18 \pm 66.72 ^{ijklmo}
	10 g/L, 10 min	378.64 \pm 71.94 ^{ijklmo}

Results were presented as result \pm uncertainty. The means in each one of the columns with a different letter were significantly different ($p<0.05$)

Table 4: Total reducing sugar and pH of dried and powdered plants

Plant	Glucose (%)	Fructose (%)	Total reducing sugar (%)	pH
Ribwort plantain	2.10±0.05	1.80±0.10	3.90±0.15 ^b	5.93±0.02 ^b
Milk thistle	2.22±0.10	2.93±0.05	5.15±0.15 ^a	5.72±0.02 ^c
Garden sorrel	2.88±0.10	2.80±0.08	5.68±0.18 ^a	6.18±0.03 ^a
Shepherd's-needle	2.84±0.07	2.80±0.08	5.64±0.15 ^a	6.21±0.01 ^a
Common poppy	2.50±0.10	2.78±0.10	5.28±0.20 ^a	6.22±0.03 ^a
Sow-thistle	2.40±0.07	3.17±0.10	5.57±0.17 ^a	6.18±0.02 ^a

Results were expressed as mean±SD (n=2). Different letters within the same column indicated significant difference (p<0.05)

Table 5: The contents of phenolic compounds in the different aqueous plant extracts (µg/g)

Plants	Aqueous extract								
	GAL	PRO	CHL	CAT	PHY	GEN	EPI	EGA	CAF
Ribwort plantain	0.24±0.1	0.1±0.05	89.5±20.5	0.03±0.01	79.5±4.5	0.2±0.02	N.D.	N.D.	0.4±0.2
Milk thistle	0.2±0.05	0.2±0.1	0.08±0.02	0.04±0.02	0.5±0.1	N.D.	N.D.	0.8±0.1	N.D.
Garden sorrel	1.3±0.3	3.8±1.5	42.5±6.5	0.06±0.02	7.5±2.5	0.04±0.02	0.04±0.01	0.08±0.01	35.0±10.5
Shepherd's-needle	0.2±0.1	0.02±0.01	0.7±0.3	0.05±0.02	1.1±0.4	0.3±0.1	N.D.	1.6±0.8	N.D.
Common poppy	0.2±0.1	1.5±0.6	0.8±0.4	0.05±0.01	10.2±5.8	0.07±0.03	N.D.	N.D.	4.5±2.3
Sow-thistle	0.1±0.05	0.1±0.02	1.3±0.4	0.04±0.01	1.2±0.3	N.D.	N.D.	0.7±0.2	N.D.
Plants	Aqueous extract								
	MYG	RUT	HYD	MYR	ELL	ISO	EPT	CUM	QUR
Ribwort plantain	0.3±0.1	0.5±0.2	1.0±0.3	0.2±0.05	N.D.	1.0±0.3	0.2±0.1	4.5±0.8	0.08±0.01
Milk thistle	0.4±0.2	2.4±0.5	N.D.	N.D.	N.D.	4.0±1.0	0.2±0.05	0.1±0.02	N.D.
Garden sorrel	0.4±0.2	23.1±4.5	1.5±0.5	0.8±0.2	79±30.5	21.6±10	0.2±0.1	3.4±1.0	0.7±0.4
Shepherd's-needle	0.4±0.2	1.4±0.5	N.D.	N.D.	N.D.	0.5±0.1	0.6±0.3	0.3±0.1	0.4±0.2
Common poppy	0.17±0.05	1.4±0.6	N.D.	0.2±0.1	N.D.	1.0±0.3	3.0±0.2	2.3±1.1	N.D.
Sow-thistle	0.04±0.01	0.02±0.01	0.02±0.01	0.5±0.1	N.D.	0.4±0.2	2.9±0.4	0.4±0.1	0.3±0.1
Plants	Aqueous extract								
	ISR	SYR	FER	QUE	SAL	TRA	ISH	KAE	CAA
Ribwort plantain	3.5±1.0	1.5±0.6	0.5±0.2	0.04±0.02	N.D.	0.08±0.01	N.D.	0.7±0.1	0.8±0.2
Milk thistle	0.04±0.01	0.08±0.03	0.9±0.1	0.02±0.01	N.D.	0.1±0.02	N.D.	0.1±0.04	N.D.
Garden sorrel	16.9±3.1	1.1±0.4	62.0±25.0	3.3±0.7	0.2±0.1	0.5±0.2	1.0±0.5	0.9±0.3	1.5±0.5
Shepherd's-needle	N.D.	N.D.	6.5±1.5	0.1±0.03	0.05±0.01	N.D.	N.D.	0.5±0.2	N.D.
Common poppy	N.D.	0.5±0.2	32.1±14.5	0.1±0.02	N.D.	0.3±0.1	N.D.	0.4±0.2	N.D.
Sow-thistle	0.3±0.1	1.1±0.5	0.7±0.2	0.01±0.005	0.05±0.01	0.5±0.2	N.D.	0.2±0.06	N.D.

GAL:Gallic Acid ; PRO: Protocatechuic Acid ; CHL: Chlorogenic Acid ; CAT: Catechin ; PHY: P-hydroxy benzoic acid (4-Hydroxy benzoic acid); GEN: Gentisic Acid; EPI: Epicatechin ; EGA: Epigallocatechin Gallate ; CAF: Caffeic Acid ; MYG: Myricetin-3-O-Galactoside ; RUT: Rutin hydrate (quercetin-3-O-rutinoside hydrate) ; HYD: 3-Hydroxybenzoic Acid ; MYR: Myricetin (myricetin-3-O-rhamnoside) ; N.D: Not Detected

ELL: Ellagic Acid; ISO: isorhamnetin 3-O-rutinoside; EPT: Epicatechin Gallate; CUM: p-Coumaric acid; QUR: Quercetin-3-D-xyloside; ISR: Isorhamnetin-3-O-glucoside; SYR: Syringetin-3-glucoside; FER: Ferulic Acid; QUE: Quercetin (quercetin-3-O-rhamnoside) ; SAL: Salicylic Acid; TRA: Trans-Resveratrol ; ISH: Isorhamnetin ; KAE: Kaempferol; CAA: o-Coumaric acid (2-Coumaric acid);

Table 6: The correlation coefficients (r) for spectrophotometric assays and acrylamide

	DPPH	ABTS	FRAP	CUPRAC	TPC	TFC	Acrylamide
DPPH	-	0.442	0.584*	0.514*	0.424	0.070	-0.197
ABTS	0.442	-	0.374	0.601*	0.801**	0.256	0.464
FRAP	0.584*	0.374	-	0.693**	0.326	0.172	0.459
CUPRAC	0.514*	0.601**	0.693**	-	0.769*	0.548*	0.435
TPC	0.424	0.801**	0.326	0.769**	-	0.711**	0.410
TFC	0.070	0.256	0.172	0.548*	0.711**	-	0.411
Acrylamide	-0.197	0.464	0.459	0.435	0.410	0.411	-

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

TFC=Total Flavonoid Content; TPC=Total Phenolic Content; DPPH=1,1-Diphenyl-2-Picrilhydrazil; CUPRAC=Cupric ion Reducing Antioxidant Capacity; FRAP=Ferric Reducing Antioxidant Power; ABTS=2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

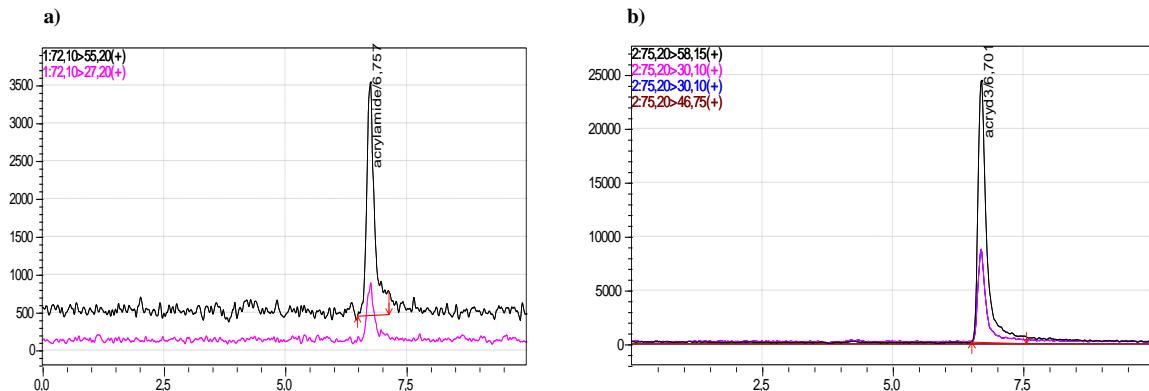


Figure 1: Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) chromatogram of acrylamide fragment ions m/z 55.0 and 27.20 in raw potato spiked at level of 60 $\mu\text{g}/\text{kg}$ acrylamide (a: left side) and Acrylamide-d3 (AAd3) fragment ions m/z 58.15 and 30.10 in raw potato spiked at level of 500 $\mu\text{g}/\text{kg}$ AAd3 (b: right side)

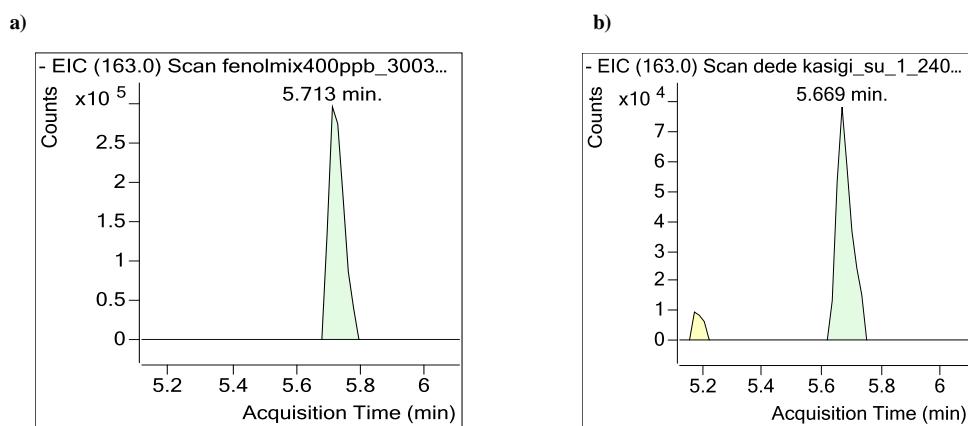


Figure 2: Liquid Chromatography-Quadrupole Time-of-Flight (LC-Q-TOF) chromatogram of p-Coumaric acid for external calibration mix at 400 ppb (a: left side) and for ribwort plantain extract (1/40 dilution) (b: right side)

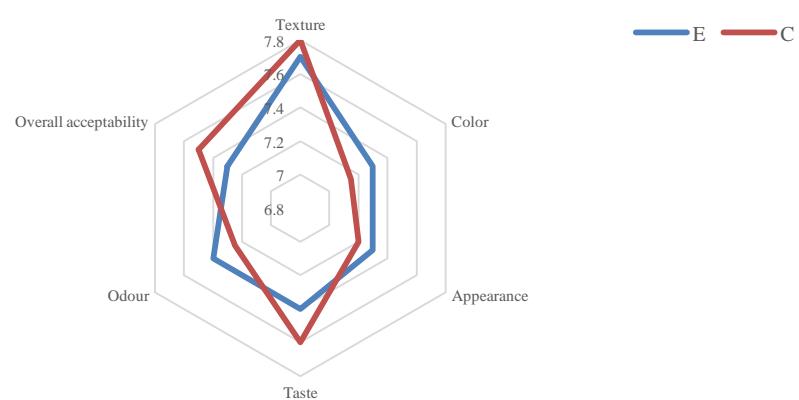


Figure 3: A radar chart for sensory analysis results of potato crisps (E: immersed in watery ribwort plantain extract at 10 g/L for 5 min and C: immersed in distilled water at 0 g/L for 5 min)

Discussion

In our study, extraction solvent changed significantly obtained results in TAC, TPC, and TFC. In a similar line, Dirar et al. (2019) measured TFC, TPC, and TAC of extracts from Sudanese medicinal plants and they mostly reported higher values of TAC, TFC, and TPC in 95% ethanol than in water. Butsat and Siriamornpun (2016) reported that TAC of 80% methanol and 80% ethanol leaf extracts of *Amomum chinense* C. were higher than distilled water extract based on DPPH, ABTS, and FRAP assays. Moreover, Sultana et al. (2009) calculated DPPH scavenging activity (%) of different medicinal plant materials with aqueous methanol (80%) and aqueous ethanol (80%) and they generally detected similar antioxidant activity in these extracts.

We found statistically significant differences between TAC, TPC, and TFC of different plants prepared in the same solvent. Kamiloglu et al. (2014) investigated TAC of Turkish herbs and spices by ABTS, DPPH, FRAP, and CUPRAC assays, and Altin et al. (2021) identified TAC of edible Mediterranean wild greens from Turkish cuisine by CUPRAC assay. Similar to our findings, they reported differences in TAC of plants.

Ayas et al. (2017) determined TAC of methanol extract of garden sorrel as $921.3 \mu\text{mol TE/g}$ fresh plant using DPPH assay. This value was higher than that was found in this study ($29.15 \pm 2.75 \mu\text{mol TE/g}$ sample) for methanol extract of garden sorrel based on DPPH assay. This difference could be related to the effect of drying, extraction technique used, and maturing stage of plant (Dhanani et al., 2017; Orphanides et al., 2013). Sulas et al. (2016) determined TAC of Sardinian milk thistle extract in acetone and water (7:3, v/v) mixture through ABTS assay as ranging between 34.5 and $54.2 \mu\text{mol TE/g}$ dry weight, which was in line with the results found in this study by ABTS assay (36.53 ± 3.20 - $63.39 \pm 7.60 \mu\text{mol TE/g}$).

In this study, TPC of methanolic ribwort plantain extract was found as $61.10 \pm 5.50 \text{ mg GAE/g}$ sample. Likewise, Bahadori et al. (2020) reported TPC of methanolic ribwort plantain extract as $45 \pm 1 \text{ mg GAE/g}$ extract. TFC of ribwort plantain was ranging from 7.20 ± 0.30 to $10.03 \pm 0.92 \text{ mg QE/g}$ sample, which was in accordance with the results ($9.6 \pm 0.03 \text{ mg QE/g}$ extract for methanol sample and $8.2 \pm 0.05 \text{ mg QE/g}$ extract for water sample) of study conducted by Bahadori et al. (2020). Furthermore, TFC of methanolic common poppy and sow-thistle extracts were found as 11.52 ± 1.02 and $13.01 \pm 1.62 \text{ mg QE/g}$ sample, respectively. Similarly, Altin et al. (2021) reported TFC of the methanolic extract of common poppy and sow-thistle from Türkiye as 4.33 ± 0.71 and $13.16 \pm 1.69 \text{ mg Catechin Equivalent (CE)/g}$ dry weight (dw), respectively.

According to the document (2017/2158) published by the EC (2017), potato products must meet the criteria of $\text{LOQ} \leq 50 \mu\text{g/kg}$ in acrylamide analysis. The LOQ ($14.59 \mu\text{g/kg}$) found is in accordance with this criterion and the level of $30 \mu\text{g/kg}$ was chosen as the reporting limit. The linear range, LOD ($4.38 \mu\text{g/kg}$), LOQ ($14.59 \mu\text{g/kg}$), repeatability (4.10%) and reproducibility (7.58%) were acceptable according to evaluation criteria. The expanded relative combined uncertainty was calculated as 19.0%.

Acrylamide levels calculated in produced potato crisps were in line with acrylamide levels of potato crisps reported (ranging from 108 to 2,180 ppb) by Mesías and Morales (2015). Application of ribwort plantain extract for 10 g/L for 5 min ensured the highest acrylamide level reduction (57%) in potato crisps. Accordingly, Zhang et al. (2007) obtained the greatest acrylamide concentration reduction (74%) in potato crisps by using bamboo leaves extract (1 g/L and 1 min) and Morales et al. (2014) obtained 62% acrylamide reduction in potato crisps by immersion sliced potatoes in the watery green tea extract (1 g/L for 1 min). In another study, El-Desouky et al. (2015) used an aqueous extract of roselle as a natural source of antioxidants and they observed 82.46% acrylamide reduction in potato crisps after soaking at 20 min with 5% extract.

In general, soaking sliced potatoes in water or watery extract for 5 min was more successful in acrylamide reduction than soaking sliced potatoes in water or watery extract for 1 min or 10 min in each concentration level. This could be related to decrease in the extraction efficiency of reducing sugars when the potato cuts were soaked in water which was used previously (Mestdagh et al., 2008). In this study, the same distilled water or aqueous extract (5 g/L or 10 g/L) was used for different application conditions (1, 5, and 10 min). Probably, differences in used plant extracts and their concentration impacted acrylamide formation so various analyses were conducted on powdered plants and their extracts so as to understand the reason behind this situation. Reducing sugars play a key role on acrylamide formation in potato crisps (Tajner-Czopek et al., 2021), therefore reducing sugar levels of powdered plants were controlled. Differences in acrylamide levels should depend on the other agents or factors because there was no statistically significant difference in the total reducing sugar (%) of the 5 plants ($p < 0.05$), apart from ribwort plantain. Ribwort plantain had a bit less total reducing sugar content compared to other plant extracts but this difference was not sufficient to explain why the lowest acrylamide level was obtained by using this extract. Jung et al. (2003) reported that lowering the pH from 7.0 to 4.0 significantly inhibited acrylamide formation (99.1%) in heated solution containing asparagine and glucose. However, pH values of studied plants were found between 5.72 and

6.22. Thus, acrylamide inhibition in potato crisps was not related to pH values of plants.

In this study, the use of plant extracts as a natural source of antioxidants did not always lead to meaningful acrylamide formation reduction in produced potato crisps. The mechanism of antioxidant on acrylamide formation has not completely clear yet. The same kind of antioxidants may behave differently in various studies owing to the differences in reaction conditions, the antioxidant concentrations, as well as the extract preparation methods (Jin et al., 2013). Published studies showed that natural antioxidants may interact with the acrylamide precursors in Maillard reaction and lipid oxidation (Ciesarová et al., 2008; Morales et al., 2014; Taeymans et al., 2004; Zhang et al., 2007). It is assumed that the reducing sugar fragments react with the antioxidant conjugated system in the Maillard reaction, thus sugar cannot unite with asparagine. During oxidation of lipids, acrylic acid from acrolein can be formed, which may form acrylamide by interacting with nitrogen sources. The antioxidants might prevent the acrolein oxidation. By this means, lower acrylamide level can be obtained in the food system (Morales et al., 2014). On the other hand, it was found that virgin olive oil phenolic extract may enhance acrylamide formation in a potato model system due to oleuropein (Jin et al., 2013).

Phenolic compounds that we found in watery plant extracts showed similarities with the result of studies done by Altin et al. 2021, Beara et al. (2012), Chrząszcz et al. (2021), Khanam et al. (2012), and Sarikurkcu et al. (2020). Altin et al. (2021) determined phenolic acids in nine wild greens from Izmir including common poppy, garden sorrel, shepherd's-needle. Nevertheless, certain phenolic acids and their amounts showed difference in the same plant compared to our results, this could be related to seasonal and extraction solvent difference. Beara et al. (2012) were collected ribwort plantain from Serbia and they detected similar phenolic acids at different concentrations in methanolic ribwort plantain extract. Although the amount of phenolic compounds detected was the highest totally in garden sorrel extract in this study, the largest acrylamide reduction was obtained when the aqueous ribwort plantain extract (10 g/L for 5 min) was used. Ribwort plantain extract contained considerably higher amount of p-hydroxy benzoic acid, chlorogenic acid, and p-Coumaric acid compared to other plant extracts. Similarly, a study done by Xu and An (2016) revealed that p-Coumaric acid may effectively reduce acrylamide formation (66.2%) in fried potato crisps. Nonetheless, Cai et al. (2014) reported that chlorogenic acid increased acrylamide formation in a model system. It was not easy to offer a specific reason for this variance since many different parameters may influence the effects of antioxidants towards acrylamide.

Furthermore, apart from phenolic compounds, other factors could be effective in acrylamide reduction. Therefore, it was very difficult to link acrylamide mitigation to a single phenolic compound in this complex system.

In terms of correlations between spectrophotometric assays, there were controversial results in the literature. Kamiloglu and Capanoglu (2015) found high correlation coefficients between their spectrophotometric assay results; however, Kim et al. (2015) observed low or negative correlation coefficients between their spectrophotometric assay values. Moreover, different results were reported in the literature regarding correlations between spectrophotometric assay results and acrylamide concentrations. Kalita et al. (2013) found a negative correlation ($r=-0.37$) between TPC content of potato powder extracts and acrylamide formation in French fries. Nonetheless, Kukurová et al. (2015) observed no significant correlation between the acrylamide content and TPC or TAC of prunes.

Conclusion

The highest acrylamide reduction was obtained in potato crisps produced from sliced potatoes immersed in ribwort plantain extract (10 g/L and 5 min). These results were promising because it was found that a wild plant extract could be used to mitigate acrylamide formation in potato crisps without adversely affecting potato crisps' sensory and color properties. This process is simple, clean and economically feasible compared to any industrial solutions. In case the consumer create demand for these greens, the rural women cooperatives could manage the supply easily. As currently the global conditions getting worse, all researches should adapt the aspects of the sustainability. Using such local resources not only makes our foods healthier but also introduce environment friendly, economically, and social cohesive solutions.

These results also indicated that the identity of phenolic compound is more important than total antioxidant activity of the system in terms of acrylamide reduction. The phenolic compound contents of plants can change with climate, season, altitude, and environment so achieving a final decision about acrylamide mitigation potential of plants is more complicated than using pure phenolic compound and a model system. Further researches are necessary to find out the reasons of conflicting acrylamide level changes in potato crisps when plant extracts are used. Also, instead of aqueous extracts, other solvent extracts serving higher TAC, TPC, and TFC could be utilized for acrylamide reduction in future studies.

Author contributions

B.A., M.G., S.G. designed the study, analysed data, and wrote the manuscript; B.A., N.A.G., M.H., A.D., A.K., R.Z.G. did experimental work; N.A.G. analysed data; N.A.G., H.T., M.A., A.T. wrote manuscript. All authors read and approved the final manuscript.

Conflicts of interest

No potential conflict of interest was reported by the authors.

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