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Polyphenols with antioxidant activity II

Research article

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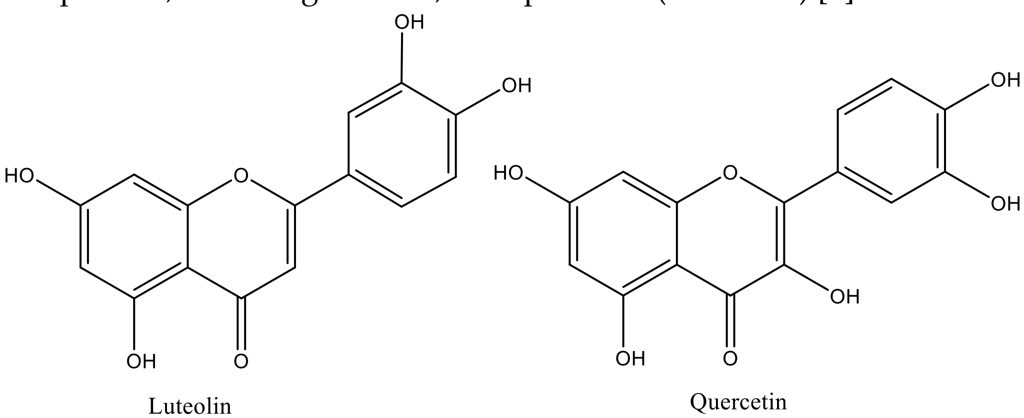
Abstract
Using ethanol extraction and the Folin-Ciocalteu technique, polyphenols were identified from some fresh and frozen natural vegetables.

Keywords: polyphenols, ethanol extraction, Folin-Ciocalteu method, UV-Vis spectrometry

1. INTRODUCTION

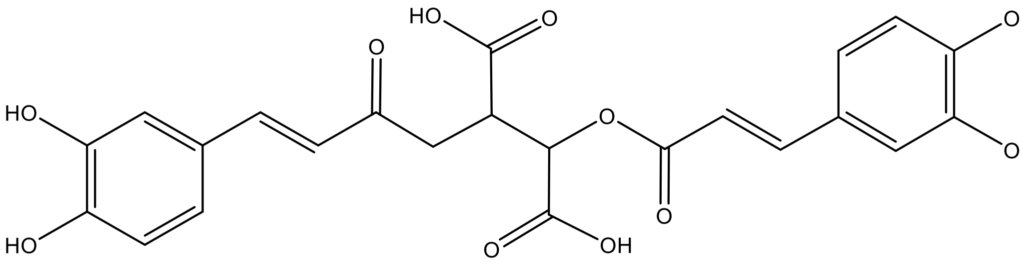
Polyphenols have anti-inflammatory and antioxidant properties, help prevent illnesses and diseases and consumers and food manufacturers are becoming more interested in them. Plants naturally contain chemicals called polyphenols, which include flavonoids and phenolic acids. The six subclasses of flavonoids (flavonols, flavones, isoflavones, flavanones, anthocyanins, and flavanols) make up the biggest group of compounds with antioxidant properties [1,2].

Peppers, a member of the Solanaceae family, are a popular vegetable due to their color, flavor, and nutritional value. Native to North and South America, they thrive in hot, dry climates and are used for medicinal and culinary purposes. Peppers are thick-walled bell-shaped vegetables with three or four lobes and vary in size and color depending on genotype or breeding period. The green color is due to chlorophyll and carotenoids, while the yellow-orange hue is due to carotene, zeaxanthin, lutein, and cryptoxanthin. The red color is due to capsanthin, capsorubin, and capsanthin 5,6-epoxides. The color of peppers also influences their taste and flavor, with red, yellow, and orange peppers being sweeter than green peppers due to higher glucose content during ripening. Bell peppers are rich sources of vitamins, phenolic or flavonoids, and bioactive compounds, with different types and quantities of bioactive compounds among different colored peppers. They were also discovered to be a good source of phenolic compounds, including luteolin, and quercetin (Scheme 1) [3].



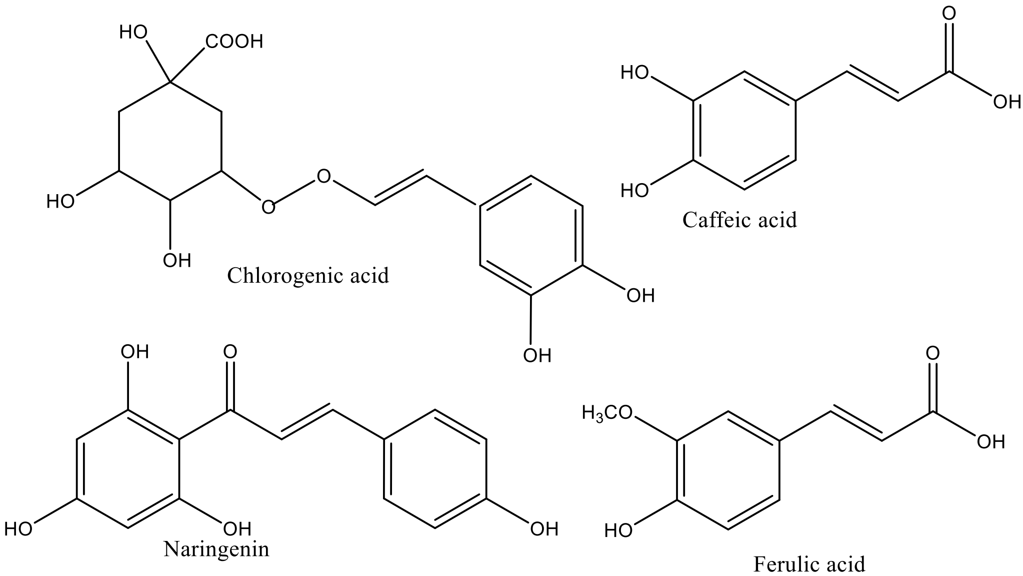
Scheme 1. Structure of some polyphenols from red peppers

Using HPLC analysis, an ethanolic antioxidant extract from leftover lettuce was investigated. The majority of the detected phenolic compounds were caffeoylquinic and caffeoyltartaric acid derivatives; of these, dicaffeoyltartaric acid (also known as chicoric acid) (Scheme 2) was the most frequently identified derivative, followed by caffeoyl tartaric acid. Furthermore, flavonoid compounds (dimethyl quercetin and luteolin) and an isomer of chlorogenic acid (3-O-caffeoylquinic acid) were discovered [4].



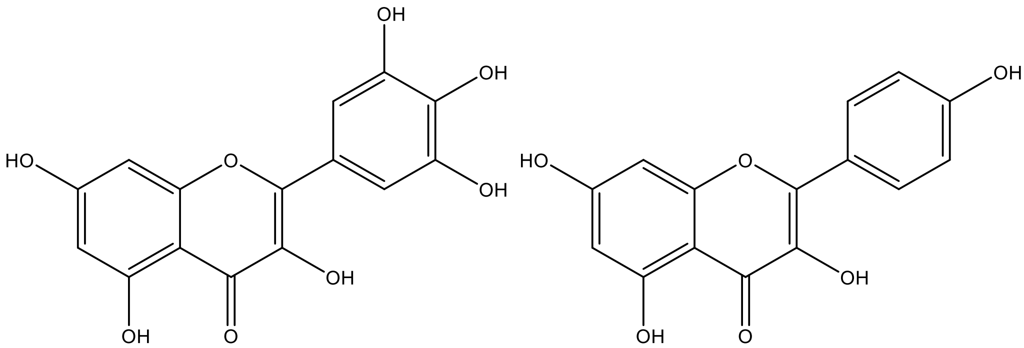
Scheme 2. Structure of chicoric acid from green lettuce

The phenolic fraction from several tomato lines and cultivars has been identified, along with the polyphenol pattern (containing flavonols and flavan). HPLC analyses and spectrophotometry were used to get the characterisation. A tomato extract's normal HPLC chromatogram shows the presence of rutin, a derivative of chlorogenic acid, caffeic acid, ferulic acid, naringenin, and its chalcone (Scheme 3). The majority of these peaks were identified by comparing their UV spectra and retention durations to those of standards[5].



Scheme 3. Structure of some polyphenols from tomatoes

One of the most popular vegetables grown worldwide is the onion (Allium cepa L.) and possess a variety of health-promoting qualities, including antioxidant, anti-mutagenic, and anti-cholesterolaemic effects. Onions contain a lot of flavonoids: myricetin, kaempferol, isorhamnetin, and quercetin mono- and diglucosides (Scheme 4). One of the most abundant foods in the human diet that contains quercetin, one of the primary flavonols, is onions. Onions have an average total quercetin content of 347 mg/kg, which is 5–10 times higher than that of other vegetables. Both the bound and free forms of quercetin, the most prevalent lavonol, are found. Additionally, trace quantities of kaempferol-glycosides are found [6]. Using HPLC and MS in positive ionization mode, the flavonoid content of honeysuckle and Artisan sweet Italian red onions was determined. The most prevalent flavonoids were discovered to be quercetin-3,4′-diglucoside and quercetin-4′-glucoside[7].



Myricetin Kaempferol

Scheme 4. Structure of some polyphenols from onion

The purpose of this study is to extract polyphenols from various fresh and frozen vegetables using ethanol and to determine the polyphenol content using the Folin-Ciocalteu method. This research is a continuation of previous studies that determined the polyphenol content of certain fruits [8].

2. MATERIALS AND METHODS

2.1. Materials

We bought ethanol, sodium carbonate, and the Folin-Ciocalteu reagent from Fluka or Aldrich. Every chemical utilized was of analytical quality and didn't require any additional purification. Deionized water was used to prepare each sample solution.

2.2. Apparatus

UV-Vis spectra of the analyzed extract were recorded from 200-800 nm by using an UV-Vis Varian Cary 50 Bio spectrophotometer.

2.3. Methods

2.3.1. Polyphenols extraction

Ten milliliters of ethanol were used to crush and finely grind one gram of the examined vegetable. It was filtered, and the resulting filtrate was used to calculate the dosage of all soluble phenols.

2.3.2. Calibration curve drawing

Six solutions with concentrations ranging from 25 to 400 μg·mL^{-1} were obtained by diluting a stock solution of gallic acid (0.4 mg·mL^{-1}), as indicated in Table 1. A 10-fold diluted Folin-Ciocalteu solution, 0.4M Na_2CO_3 solutions, and 0.1 mL of each solution have been added. The absorbances at 765 nm were measured after 15 minutes.

Table 1. The absorbances of gallic acid solutions

A_{765}/nm	Concentration/μg·mL^{-1}
0.154	25
0.286	50
0.413	100
0.788	200
1.085	300
1.571	400

The calibration curve (Figure 1) has been obtained.

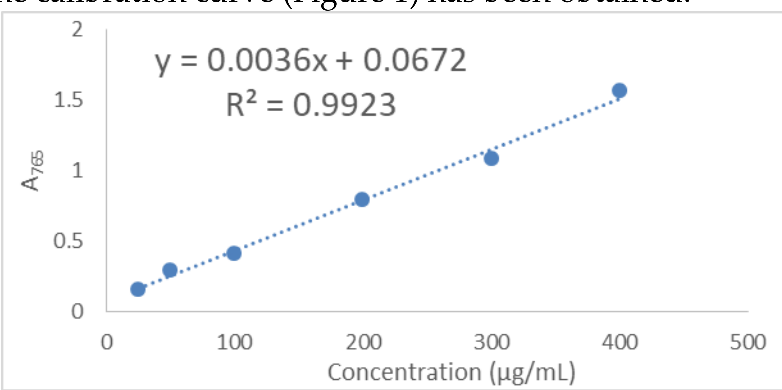


Figure 1. Gallic acid calibration curve

2.3.3. Total soluble phenols dosage

The Folin-Ciocalteu reagent was used to measure the total phenolic content of the ethanolic extract. Deionized water was used to dilute the Folin-Ciocalteu reagent 1:9. One milliliter of diluted Folin-Ciocalteu reagent, one milliliter of ethanolic extract, and one milliliter of 0.4 M Na_2CO_3 solution were combined. For fifteen minutes, the resulting mixture was allowed to rest. Lastly, the absorbances at 765 nm were measured and the UV-Vis spectra were recorted.

3. RESULTS AND DISCUSSION

One of the more well-known classes of polyphenols is flavonoids. With the exception of algae and mushrooms, they are most prevalent in edible plant items, especially fruits and vegetables. Green-leafed, yellow, and red vegetables (such as onions, cabbages, broccoli, cauliflower, Brussels sprouts, pulse seeds, tomatoes, and peppers), fruit (such as grapefruits, oranges, berries, red and black currants, dark grapes, apples, and aronia), tea (particularly green tea), and red wine are foods high in flavonoid compounds [9-11]. For the dosage of the soluble phenols we used the following vegetal materials: red peppers, tomatoes, green lettuce, yellow onions, and red onions.

Equation (1) was used to determine the total phenolic content, which was represented in terms of gallic equivalent (mg of gallic acid equivalent/g of extract):

$$T = C \times \frac{V}{M} \qquad (1)$$

T= total phenolic content ($mg \cdot g^{-1}$ extract), named EAG;
C= gallic acid concentration ($mg \cdot mL^{-1}$)
M= mass of the analyzed sample
V= sample volume

The total phenolic content of the analyzed vegetables is presented in Table 2.

Red peppers are the most abundant source of antioxidants, as shown in Table 2 and Figure 2. The tomato, which stands for a tiny amount of natural antioxidants, is at the other extreme. Green lettuce, which is renowned for its antioxidant qualities, follows red pepper.

Additionally, we can see that some of the antioxidant qualities of veggies are lost when they are frozen. The concentration of antioxidants in fresh green lettuce is more than half that of frozen green lettuce.

Table 2. The absorbances of analysed solutions

No.	Analysed fruits	A_{765}/ nm	Concentration/ µg·mL^{-1}	Phenols amount/ µg·g^{-1}
1	Red pepper	1,71	4,209	42,09
2	Green lettuce	0,53	1,33	13,3
3	Frozen green lettuce	0,23	0,59	5,9
4	Red onion	0,37	0,94	9,4
5	Frozen red onion	0,32	0,81	8,1
6	Tomato	0,29	0,74	7,4
7	Frozen tomato	0,18	0,47	4,7
8	Yellow onion	0,34	0,86	8,6
9	Frozen yellow onion	0,24	0,62	6,2

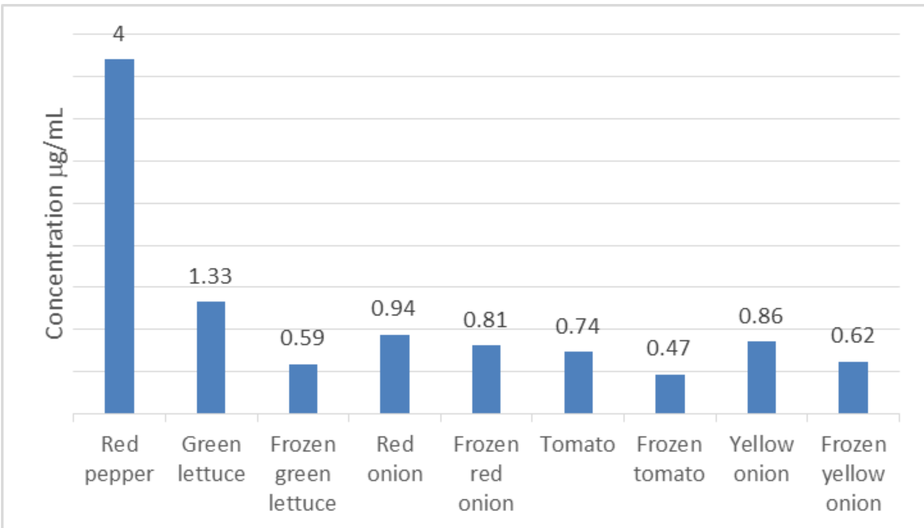


Figure 2. The polyphenol content of the analyzed samples

4. CONCLUSION

The Folin-Ciocalteu method was used to measure the polyphenol content in red peppers, tomatoes, green lettuce, yellow onions, and red onions. Tomatoes have the least quantity of polyphenols, while red pepper have the most. The quantity of polyphenols is reduced by freezing.

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