Research Article

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Simultaneous extraction and quantification of hydrophilic and lipophilic antioxidants in *Solanum lycopersicum* L. varieties marketed in Saudi Arabia

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Abstract: Several varieties of *Solanum lycopersicum* L. are consumed in Saudi Arabia, including the most expensive Ramis tomato, which is claimed to be rich in antioxidants. However, there is a lack of studies on the extraction and quantification of antioxidants in tomatoes marketed in Saudi Arabia. Therefore, this study aimed to develop a straightforward method to extract and quantify phenolics and carotenoids in tomatoes consumed in Saudi Arabia. The developed method used glass coverslips for homogenisation and a methanol-petroleum ether mixture for extraction. In 15 investigated varieties, antioxidants were abundantly present in the skin part (2.0 ± 0.72 mg/g of phenolics and 229.50 ± 21.10 μg/g of carotenoids) compared to the pulp (0.67 ± 0.20 mg/g of phenolics and 107.70 ± 21.10 μg/g of carotenoids). Generally, the Saudi varieties contained higher amounts of antioxidants than the imported varieties. The antioxidant content of the Ramis cultivar depends on the cultivation location. For instance, Ramis Awjam shows high amounts of phenolics (2.72 mg/g in the skin extract and 0.63 mg/g in the pulp) and carotenoids (338.04–388.41 μg/g in the skin and 173.93–205.75 μg/g in the pulp). This study provides valuable insights that can assist in selecting the cultivar and location of tomatoes with the maximum antioxidant quantities.

Keywords: tomato, gallic acid, phenolics, carotenoids, lycopene

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1 Introduction

Solanum lycopersicum L., commonly known as tomato, is a crucial crop classified botanically as a fruit but typically consumed as a vegetable [1,2]. This popularity is due to their excellent flavour and nutritional value [3]. They are a rich source of numerous essential vitamins, including vitamins C, B-complex, A, E, and K [2]. They also provide essential minerals like calcium, magnesium, phosphorus, and potassium [2]. Trace elements such as iron, iodine, zinc, fluorine, and copper are also found in tomatoes, making them a valuable dietary source of vitamins and minerals [2,4].

Tomatoes possess bioactive phytochemicals, including phenolic and carotenoid compounds, that provide antioxidant, anti-inflammatory, and anti-proliferative properties, contributing to the prevention of chronic conditions like cardiovascular diseases, cancer, and diabetes [5,6]. Key antioxidants, including phenolic and carotenoid compounds, reduce the risk of degenerative diseases by decreasing oxidative stress induced by reactive oxygen species (ROS) [2,5,7]. They also play a role in reducing the risk of cataracts [8]. Their high water content and fibre also aid digestion and support weight loss [2].

A wide range of antioxidant phytochemicals, mainly phenolic and carotenoid compounds, are present in tomatoes. Carotenoids, specifically α-carotene, β-carotene, lycopene, and zeaxanthin, are some of the most crucial compounds found in tomatoes [2,6,9]. Furthermore, tomatoes contain bioactive phenolic and polyphenolic compounds such as rutin, naringenin, naringenin chalcone, quercetin, kaempferol, gallic acid, ellagic acid, chlorogenic acid, ferulic acid, and caffeic acid, which are present in tomatoes at lower concentrations than carotenoids and contribute to their nutritional and health-promoting qualities [3,5,10].

The type and level of phytochemicals are influenced by several factors such as ripeness, cultivar type, geographical region, and agronomic conditions [11,12]. The degree of

ripeness directly affects the concentration of lycopene [13]. Lycopene concentration in the peel is higher than in pulp [14], whereas β-carotene levels are significantly influenced by the cultivar, as demonstrated in the Tudor-Radu et al. study [15].

Tomatoes are a significant economic commodity in Saudi Arabia, with annual production exceeding 180 million metric tonnes in 2022, with 119 million tonnes (63.9%) produced in Asia [16]. The tomato varieties are cultivated locally (Kharj, Qassim, Taif, Alhasa, Haradh, Ramis, etc.) or imported from international producers. The Ramis tomato is highly regarded as an important cultivar due to its high-quality antioxidant content. The tomato varieties marketed in Saudi Arabia, including the expensive Ramis tomato, might vary in terms of the abundance of phenolics and carotenoids and, consequently, their bioactivity and health benefits.

The difference in polarity between phenolic (soluble in polar solvents such as methanol) and carotenoid antioxidants (soluble in non-polar solvents such as petroleum ether) makes it challenging to extract both classes with a simple extraction method [17]. Conventional solvent extraction techniques are used to extract phytochemicals from plant materials [18]. However, they have drawbacks, such as consuming large volumes of organic solvents and having a long extraction process [19]. Recently, alternative eco-friendly methods like ultrasound-assisted extraction (USE), microwave-assisted extraction (MAE), and supercritical fluid extraction offer advantages like enhanced extraction yields, reduced solvent consumption, and decreased extraction time [18,19]. However, the implementation of these methods requires expensive instruments, human resources, preparation, and homogenisation and may affect the properties of extracted phytochemicals [20].

There is a lack of studies on the simultaneous extraction and quantification of hydrophilic and lipophilic antioxidant compounds in tomatoes marketed in Saudi Arabia, requiring a more efficient extraction method. Therefore, this study aims to develop a simplified, one-step, cost-effective, and fast method for simultaneous extraction and quantification of phenolics and carotenoids in tomato varieties marketed in Saudi Arabia, with a focus on the expensive Ramis tomato.

2 Materials and methods

2.1 Solvents and chemical reagents

Methanol, petroleum ether, Folin–Ciocalteu reagent, and high-purity grade (≥98%) gallic acid were obtained from Sigma-Aldrich (Darmstadt, Germany). Sodium carbonate was obtained from PanReac AppliChem (Barcelona, Spain). A Pure Lab Ultra water system (ELGA, High Wycombe, U.K.) was used to prepare deionised water.

2.2 Samples collection and preparation

The most common tomato (*S. lycopersicum*) varieties were purchased from the local market in the eastern province of Saudi Arabia. Fourteen round, medium-sized tomato samples (Alhasa, Ramis Awjam, Taif, Kharj, Haradh, Ramis Abumaan, Ramis original, Ramis Jarudiyah, Syria, Jordan, Tabuk, Egypt, Turkey, Qassim) and one oblong small-sized tomato sample (Haradh candy) were purchased and stored at −40°C. After freezing the samples, the skin was easily separated from the pulp using forceps and scissors. One gram of each tomato pulp and skin was transferred to clean 50 mL centrifuge tubes labelled with the codes T1p–T15p (for pulp samples) and T1s–T15s (for skin samples).

2.3 Development of extraction method

T1p and T1s samples (Alhasa) were utilised to develop a fast, cost-effective, and simple extraction method of both phenolics and carotenoids. The samples were extracted in triplicate with 10 mL methanol (Alhasa pulp-M and Alhasa skin-M), 10 mL petroleum ether (Alhasa pulp-PE and Alhasa skin-PE), or 10 mL methanol plus 10 mL petroleum ether (Alhasa pulp-M + PE and Alhasa skin-M + PE). Glass rectangular coverslips were added in each tube to help cut and homogenise pulp and skin while vortexing the tubes for 4 min (Corning® LSE™ Vortex Mixer). Subsequently, the tubes were centrifuged (Kubota Corporation, Japan) at 3,600 rpm for 10 min to remove solid particles and separate the organic solvent layers. The methanolic layer was used to determine the phenolic content, while the petroleum ether layer was used to determine the carotenoid content. After method development, the fast, cost-effective, simple method was applied to the fifteen tomato varieties.

2.4 Determination of total phenolic content

The total phenolic content was measured using the Folin–Ciocalteu procedure with minor modifications [21]. Briefly, 360 µL from the methanolic layer of each sample was mixed

with 7.11 µL of distilled water and 450 µL of Folin–Ciocalteu reagent in test tubes protected with aluminium foil. After 2 min, 1.35 mL of 15% aqueous sodium carbonate solution was added, and the tubes were closed and placed in a water bath in the dark at a temperature of 37°C for 30 min. The absorbance was measured with a Shimadzu UV-1800 spectrophotometer (Tokyo, Japan) at 765 nm. Total phenolic content was reported as mg of gallic acid equivalent (mg GAE) per gram of tomato sample using the linear regression equation of the plotted calibration curve (dilutions of 15, 30, 180, 300, 600, and 900 mg GA/L in methanol).

2.5 Determination of carotenoid content

Carotenoids (α-carotene, β-carotene, lycopene, and zeaxanthin) were quantified by a Shimadzu UV-1800 spectrophotometer (Tokyo, Japan) following the procedure described by Rodriguez-Amaya and Kimura [22]. The concentration of each carotenoid was calculated in petroleum ether using the following equation:

$$C = A/(L \times \varepsilon) = \text{Absorbance/Absorbance coefficient},$$

C is the concentration of the measured solution, A is the absorbance of the measured solution, L is the optical path length (1 cm), ε is the absorbance coefficient (2,800 for α-carotene at 444 nm, 2,592 for β-carotene at 450 nm, 3,450 for lycopene 470 nm, and 2,348 for zeaxanthin at 449 nm; in petroleum ether).

2.6 Statistical analysis

SPSS V 22.0 and GraphPad Prism 8 were used to analyse the data and create the graphs. The results are expressed as the mean ± standard deviation (SD) from at least three independent experiments. All data were analysed with a P-value of <0.05 considered significant.

3 Results

3.1 Impact of extraction method on phenolic and carotenoid content

Methanol and petroleum ether solvents were selected for extraction due to their negligible miscibility, enabling the extraction of lipophilic and hydrophilic compounds in one step with two separate layers. Additionally, methanol and petroleum ether have been reported as efficient solvents for extracting and quantifying phenolics and carotenoids, respectively [17,22]. Petroleum ether (upper layer) contained carotenoids, while the lower layer of methanol contained phenolics. The amount of the extracted phenolics with methanol and petroleum ether mixture was comparable to that extracted separately with methanol (Figure 1). Similarly, the amount of carotenoids (α-carotene, β-carotene, lycopene, and zeaxanthin) extracted with the methanol and petroleum ether mixture was comparable to that extracted separately with petroleum ether (Figure 2). The addition of two rectangular glass coverslips into the tubes repeatedly cut the tomato skin and pulp samples during the vortex of the samples, resulting in size reduction and representing a very efficient method for homogenisation.

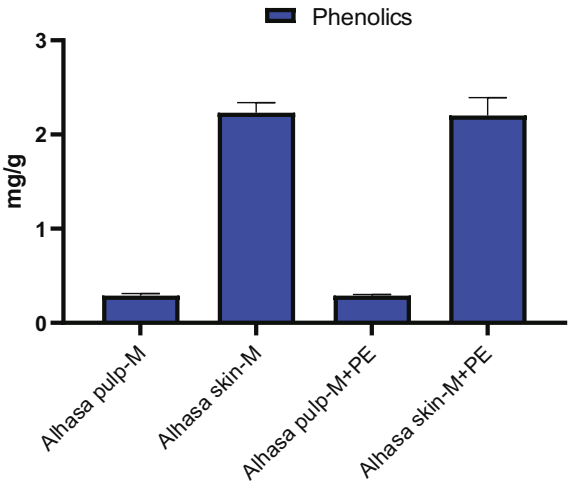

Figure 1: The impact of the extraction method on phenolic content.

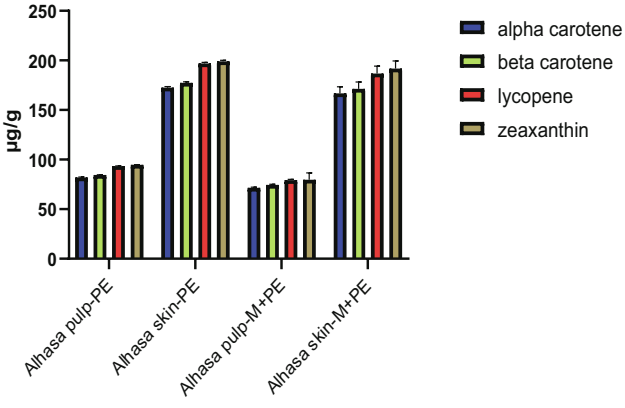

Figure 2: The impact of the extraction method on carotenoid content.

3.2 Quantification of phenolics and carotenoids in tomato varieties

3.2.1 Descriptive analysis

The mean ± SD (μg/g carotenoids; mg/g phenolics) (N = 30) for α-carotene 156.05 (±91.84), β-carotene 161.24 (±93.83), lycopene 166.27 (±105.45), zeaxanthin 190.86 (±110.95), and phenolic content 1.35 (±0.86) were observed with a range (minimum–maximum) of 50.4–401.8, 52.2–412.0, 51.4–448.6, 58.4–461.6, and 0.3–3.4, respectively. The maximum and mean quantities for these samples (N = 30) constructed a descending order for phenolics and carotenoids as follows: phenolics (3.4 and 1.347) > zeaxanthin (461.6 and 190.86) > lycopene (448.6 and 166.27) > β-carotene (412.0 and 161.12) > α-carotene (401.8 and 156.05).

3.2.2 Mean comparison

The tomato samples were analysed for the mean differences between the pulp and skin fruit parts in terms of phenolic and carotenoid content in individual samples. The computation of mean for α-carotene in the pulp part of tomato samples (N = 15) revealed the mean value of (μg/g); Alhasa (71.55), Ramis Awjam (173.93), Taif (132.44), Kharj (115.71), Haradh (161.61), Ramis Abuman (73.15), Ramis original (104.40), Ramis Jarudiyah (51.01), Syria (66.73), Jordan (81.73), Tabuk (50.42), Egypt (114.11), Turkey (69.05), Haradh candy (125.180), and Qassim (103.63). The descending order for α-carotene quantity in these tomato pulps is as follows: Ramis Awjam > Haradh > Taif > Candy > Kharj > Egypt > Ramis original and so on. The skin part of these tomato samples (N = 15) showed the following α-carotene mean values of (μg/g): Alhasa (166.67), Ramis Awjam (338.04), Taif (332.86), Kharj (401.79), Haradh (327.68), Ramis Abuman (256.19), Ramis original (182.02), Ramis Jarudiyah (74.76), Syria (159.46), Jordan (134.17), Tabuk (152.20), Egypt (147.02), Turkey (121.79), Haradh candy (200.00), and Qassim (192.26). The descending order for α-carotene quantities in the skin part of the tomato samples is Kharj > Ramis Awjam > Taif > Haradh > Ramis Abumaan > Candy > Qassim.

For the β-carotene amount in tomato pulp, the mean (N = 15) observed were (μg/g) Alhasa (74.40), Ramis Awjam (177.21), Taif (135.80), Kharj (117.54), Haradh (169.69), Ramis Abuman (76.26), Ramis original (109.50), Ramis Jarudiyah (54.21), Syria (68.87), Jordan (84.23), Tabuk (52.15), Egypt (118.31), Turkey (70.92), Haradh candy (129.24), and Qassim (107.51). The descending order for the sample with the highest amount of β-carotene in the pulp is Ramis Awjam > Haradh > Taif > Egypt > Kharj. The amount of β-carotene in the skin part (N = 15) showed the mean values (μg/g): Alhasa (171.36), Ramis Awjam (345.87), Taif (338.48), Kharj (411.97), Haradh (340.99), Ramis Abuman (262.86), Ramis original (188.85), Ramis Jarudiyah (79.03), Syria (166.35), Jordan (140.43), Tabuk (157.28), Egypt (157.54), Turkey (127.25), Haradh candy (204.48), and Qassim (195.15). The descending order for the samples with the highest quantities of β-carotene is Kharj > Ramis Awjam > Haradh > Taif > Ramis Abumaan.

The spectrophotometric quantification of lycopene content in the pulp part (N = 15) of the tomato fruits showed the following concentrations (μg/g): Alhasa (79.28), Ramis Awjam (205.75), Taif (151.21), Kharj (134.88), Haradh (173.96), Ramis Abuman (79.18), Ramis original (111.06), Ramis Jarudiyah (51.35), Syria (75.99), Jordan (90.97), Tabuk (56.96), Egypt (125.27), Turkey (78.07), Haradh candy (98.31), and Qassim (81.84). The samples with the highest amount of lycopene are Ramis Awjam > Haradh > Taif > Kharj > Egypt. For the lycopene content in the skin part of the tomato fruits, the mean observed for individual samples was (μg/g) Alhasa (186.71), Ramis Awjam (388.41), Taif (391.30), Kharj (448.60), Haradh (355.85), Ramis Abuman (284.25), Ramis original (192.75), Ramis Jarudiyah (76.23), Syria (174.83), Jordan (144.78), Tabuk (166.710), Egypt (145.75), Turkey (132.85), Haradh candy (156.04), and Qassim (149.08). The descending order for the samples with the highest concentrations is Kharj > Taif > Ramis Awjam > Haradh > Ramis Abumaan.

The zeaxanthin content in the pulp part of the tomato fruits revealed the mean (μg/g) as follows: Alhasa (79.64), Ramis Awjam (199.11), Taif (152.26), Kharj (131.89), Haradh (185.62), Ramis Abuman (85.11), Ramis original (123.65), Ramis Jarudiyah (60.260), Syria (77.09), Jordan (94.410), Tabuk (58.42), Egypt (132.24), Turkey (79.50), Haradh candy (202.94), and Qassim (164.68). The samples with the highest zeaxanthin content may be ordered as Haradh candy > Ramis Awjam > Haradh > Qassim > Taif. The skin part of the tomato fruits showed the following mean zeaxanthin values (μg/g): Alhasa (191.72), Ramis Awjam (388.34), Taif (380.32), Kharj (461.60), Haradh (381.53), Ramis Abuman (295.93), Ramis original (211.24), Ramis Jarudiyah (88.09), Syria (186.120), Jordan (157.01), Tabuk (176.11), Egypt (175.18), Turkey (142.25), Haradh candy (329.07), and Qassim (334.61). The descending order of the samples with the highest zeaxanthin content is Kharj > Ramis Awjam > Taif > Haradh > Qassim.

The content of phenolics in the pulp part of the tomato fruit exhibited the following mean values (mg/g): Alhasa (0.29), Ramis Awjam (0.63), Taif (0.41), Kharj (0.69), Haradh (0.74), Ramis Abuman (0.60), Ramis original (0.91), Ramis Jarudiyah (0.75), Syria (0.40), Jordan (0.60), Tabuk (0.84), Egypt (0.99), Turkey (0.60), Haradh candy (0.61), and Qassim (0.95). The samples with the largest amount of phenolics can be ordered as Egypt > Qassim > Ramis original > Tabuk >

Ramis Jarudiyah. The quantity of phenolics in the skin part of the tomato samples was observed with the following mean (μg/g): Alhasa (2.20), Ramis Awjam (2.72), Taif (0.64), Kharj (1.95), Haradh (1.36), Ramis Abuman (2.33), Ramis original (2.71), Ramis Jarudiyah (2.48), Syria (1.26), Jordan (1.68), Tabuk (3.41), Egypt (2.74), Turkey (1.78), Haradh candy (1.65), and Qassim (1.48). The descending order for the skin part of the tomato samples was found to be Tabuk > Egypt > Ramis Awjam > Ramis original > Ramis Al Jarudiyah.

The total concentrations of the antioxidant phytochemicals in the skin and pulp parts for each tomato cultivar are presented in Table 1. The highest total amount of carotenoids in each cultivar was detected in the Kharj and Ramis Awjam tomatoes, followed by Haradh and Taif tomatoes. In contrast, Ramis Jarudiyah and Turkey tomatoes contained the lowest amount of carotenoids, followed by Tabuk and Jordan tomatoes. For phenolics, the Tabuk tomato contained the highest concentrations, followed by Egypt, Ramis original, Ramis Awjam, and Ramis Jarudiyah tomatoes. In contrast, the lowest concentration of phenolics was measured in the Taif tomato.

3.2.3 Principle component analysis (PCA)

The scree plot suggested two components, and the component loading for the different tomato samples revealed a cumulative variance of 88.71%. The first component (PC1) accounts for the majority of the variance (69.79%), while the second component (PC2) explains 18.92%. There is a strong correlation between the four carotenoids (α-carotene, β-carotene, lycopene, and zeaxanthin) in PC1 and a moderate correlation between the phenolics and this component (Figure 3). A stronger correlation was observed for phenolics with PC2 than PC1. Hence, phenolics and carotenoids exhibited a moderate correlation in some samples but are not necessarily correlated in all samples. The Kaiser–Meyer–Olkin (KMO) and Bartlett's tests showed significant data ($P = 0.05$) with χ^2 value of 496.08 (Table 2).

3.2.4 Pearson's correlation analysis

Pearson's analysis further confirmed the PCA results of the stronger correlation between the carotenoids as compared to the phenolics. A significant correlation ($P < 0.001$) was observed between the carotenoid pairs (α-carotene, β-carotene, lycopene, and zeaxanthin), demonstrating a strong linear relationship and consistent co-occurrence. In contrast, the phenolic compounds exhibited much lower correlation values, indicating a more independent distribution. This suggests that the tomato samples that are highly enriched with carotenoids (α- and β-carotene, lycopene, and zeaxanthin) are not necessarily high in phenolics, and vice versa. The data for Pearson's correlation are illustrated in Table 3.

3.2.5 K-mean cluster analysis with analysis of variance (ANOVA)

The samples ($N = 30$) were distributed into five groups as clusters (samples): 1 (5), 2 (5), 3 (2), 4 (16), and 5 (2). The remarkable clusters herein were clusters 2 and 5, where the presence of all the phytochemicals (phenolics and

Table 1: The total concentrations of the antioxidants in each tomato cultivar

Sample cultivar	α-Carotene	β-Carotene	Lycopene	Zeaxanthin	Phenolics
Alhasa	238.21	245.76	265.99	271.37	2.49
Ramis Awjam	511.96	523.08	594.15	587.45	3.35
Taif	465.30	474.28	542.51	532.58	1.05
Kharg	517.50	529.51	583.48	593.48	2.64
Haradh	489.29	510.67	529.81	567.15	2.09
Ramis Abuman	329.35	339.12	363.43	381.03	2.94
Ramis original	286.43	298.35	303.82	334.89	3.61
Ramis Jarudiyah	125.77	133.23	127.58	148.35	3.23
Syria	226.19	235.21	250.82	263.20	1.66
Jordan	215.89	224.67	235.75	251.42	2.28
Tabuk	202.62	209.43	223.67	234.53	4.25
Egypt	261.13	275.85	271.01	307.42	3.73
Turkey	190.83	198.17	210.92	221.75	2.38
Haradh candy	325.18	333.72	254.35	532.01	2.25
Qassim	295.89	302.66	230.92	499.29	2.42

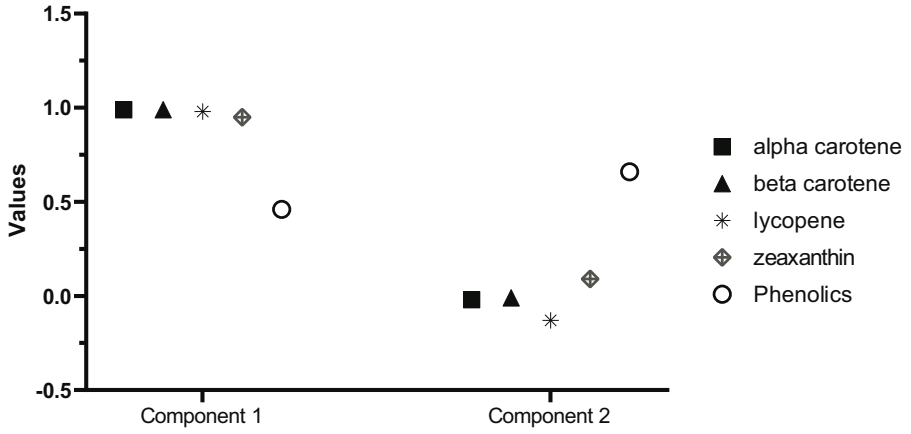

Figure 3: PCA rotated component matrix of tomato samples.

Table 2: PCA, KMO, and Bartlett's test

Factor	PC1	PC2	KMO and Bartlett's test		
α-Carotene	0.99	−0.02	KMO measure of sampling adequacy		0.53
β-Carotene	0.99	−0.01	Bartlett's test of sphericity	χ^2	496.08
Lycopene	0.98	−0.13		Df	15
Zeaxanthin	0.95	0.09		Sig.	0.00
Phenolics	0.46	0.66			
Individual variance (%)	69.79	18.92			
Cumulative variance (%)	69.79	88.72			

carotenoids) was observed in these samples (Figure 4). However, cluster 5 exhibited the samples with high quantities of phenolics and carotenoids together, while cluster 2 showed comparatively low amounts of phenolics and carotenoids. Cluster 5 consisted of two samples of Ramis Awjam and Kharj skin parts and was observed with the highest concentrations of phenolics and carotenoids. The samples grouped in cluster 2 were the Alhasa skin part, Ramis Abuman skin part, Ramis original skin part, Haradh candy skin part, and Qassim skin part. Cluster 3 (high-carotenoid group) represents the samples with the second highest quantities of carotenoids, following cluster 5, but these samples contain low amounts of phenolics. The samples observed in cluster 3 were the Taif skin part and the Haradh skin part. Cluster 1 (high-phenolic group) revealed the presence of the highest amount of phenolics among these clustered samples. The samples in this cluster, i.e., Ramis Jarudiyah skin part, Jordan skin part, Tabuk skin part, Egypt skin part, and Turkey skin part, contained limited amounts of carotenoids. The remaining samples (16) with the lowest concentrations of phenolics and carotenoids were grouped in cluster 4.

The data for the K-mean analysis are shown in Table 4. This data align with the mean analysis conducted for these samples, where the samples from Ramis Awjam, Kharj, Taif, and Haradh were found to be the richest origins,

Table 3: Bivariate Pearson's correlation analysis for different origin tomato samples

Pearson's correlation		α-Carotene	β-Carotene	Lycopene	Zeaxanthin	Phenolics
α-Carotene	Pearson's r	1				
	P value	—				
β-Carotene	Pearson's r	1.00	1			
	P value	0.00	—			
Lycopene	Pearson's r	0.98	0.98	1		
	P value	0.00	0.00	—		
Zeaxanthin	Pearson's r	0.96	0.96	0.90	1	
	P value	0.00	0.00	0.00	—	
Phenolics	Pearson's r	0.38	0.39	0.35	0.36	1
	P value	0.03	0.03	0.05	0.04	—

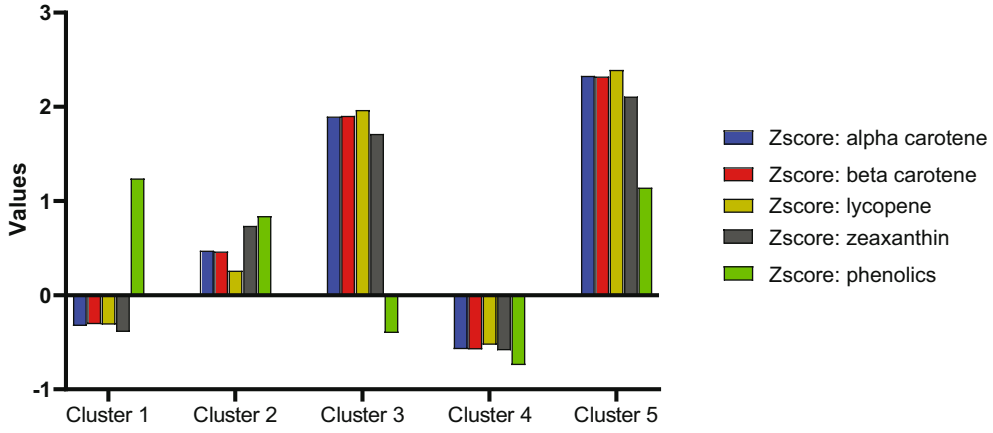

Figure 4: K-mean analysis with cluster loading for the dependent variables in tomato samples.

showing the presence of the highest concentrations of phenolics and carotenoids. The ANOVA for the tested data showed a significant difference for the phytochemical classes in these samples ($P = 0.00$) with F-values mentioned in Table 4.

4 Discussion

The objective of the present study is to create a straightforward, one-step procedure for simultaneously extracting phenolics and carotenoids in tomato cultivars sold in Saudi Arabia. Hence, it is worth mentioning that this study did not conduct any biological assays or clinical trials to correlate the antioxidant content with the health benefits. However, the relationship between the levels of phenolic and carotenoid antioxidants and health benefits has been reported in several studies. The health benefits include contributing to the prevention of cancer and chronic conditions such as diabetes and cardiovascular diseases [5,6] and reducing the risk of cataracts [8] and degenerative diseases by decreasing oxidative stress induced by ROS [2,5,7].

Table 4: K-mean cluster analysis of tomato samples with *P* and *F* values

Factors	*F* value	*P* value	Clusters	Samples
Z score: α-carotene	38.58	0.00	1	5
Z score: β-carotene	37.78	0.00	2	5
Z score: lycopene	33.75	0.00	3	2
Z score: zeaxanthin	28.20	0.00	4	16
Z score: phenolics	23.69	0.00	5	2
			Total	30

The efficiency of the simple one-step extraction with a methanol and petroleum ether mixture for both phenolics and carotenoids was comparable to that with methanol and petroleum ether performed in two separate steps. The use of this solvent mixture removed the requirement for evaporation and re-solubilisation of the samples in appropriate solvents for compound determination by spectrophotometry. Moreover, adding the rectangular glass coverslips in the tubes excluded the homogenisation step, which is usually required before extraction [23]. This simplified and accelerated extraction and quantification of phenolics and carotenoids in 30 tomato samples marketed in Saudi Arabia. Although efficient methods such as MAE and USE have been developed for the extraction of phenolic and carotenoid compounds [24,25], the method developed in this study has the advantage of simplicity when advanced instruments are inaccessible. Additionally, the inclusion of glass coverslips removes the need for the use of an electric blender for homogenisation before MAE and USE. However, this method will benefit from including less toxic solvents like ethanol. The main issue with using other solvents in this method is their miscibility, complicating the separation of the carotenoid-containing and phenolic-containing layers. Moreover, the standard methods for the quantification of carotenoids by spectrophotometer depend on the absorbance coefficient in specific solvents, such as petroleum ether. Therefore, the flexibility for the selection of solvents is complicated by the abovementioned factors, although it is not irresolvable.

In terms of efficiency, the developed method is comparable to previously reported methods for the extraction of antioxidants from tomatoes. In a study performed by Chada et al. in 2022, the lycopene content in the extract obtained by MAE was 59.66 µg/g extract [24], while in our study, the lycopene content in different tomato varieties

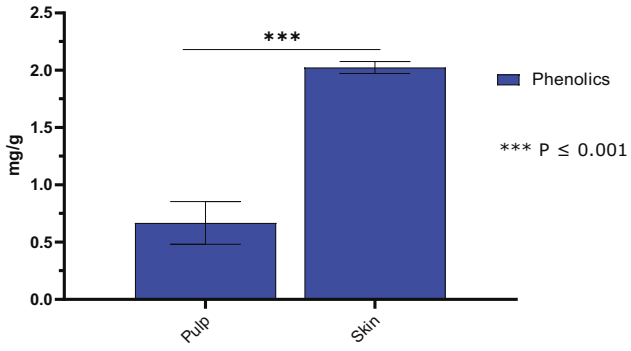

Figure 5: Phenolic concentrations in the pulp and skin of tomatoes, compared using an independent samples *t*-test. The data represents the mean ± standard error mean.

ranged between 127.58 and 594.15 µg/g. In another study conducted by Sengkhamparn and Phonkerd in 2020, the β-carotene content (117.95 µg/g) in the tomato extract obtained by USE [25] was lower than the β-carotene amount in tomato extracts produced by the method utilised in this study (133.23–529.51 µg/g).

Antioxidant phytochemicals (phenolics and carotenoids) were abundantly present in the skin part (2.0 ± 0.72 mg/g of phenolics and 229.50 ± 21.10 µg/g of carotenoids) compared to the lower amounts (0.67 ± 0.20 mg/g of phenolics and 107.70 ± 21.10 µg/g of carotenoids) in the pulp part (Figures 5 and 6). This is consistent with most previous studies that reported the presence of high amounts of phenolics and carotenoids in the skin compared to the pulp of tomato fruit [12,23,26]. For example, in a previous study on Indian tomatoes, the maximum lycopene amount measured by spectrophotometry was 143 µg/g in tomato skin, while the pulp contained only 67 µg/g of this carotenoid [27]. Another study on tomatoes marketed in Croatia reported lower amounts of lycopene in Croatian tomatoes compared to Indian tomatoes [12]. However, the lycopene amount in the skin of this cultivar (53 µg/g) was still higher than the amount in the pulp part (16 µg/g). Similarly, the skin of tomatoes has been reported to contain the highest levels of total phenolic content compared to the pulp and seed parts [23,27,28]. The phenolic content in the skin of seven Indian tomato cultivars was reported to range from 0.28 to 0.44 mg GAE/g compared to 0.13–0.17 mg GAE/g in the pulp [29]. Bianchi et al. [23] reported that the ethanolic extract of tomato skin contains over 0.5 mg GAE/g of phenolic compounds, while the pulp of the same cultivar contains less than 0.2 mg GAE/g. The larger quantities of antioxidants in the skin part compared to other parts have also been proven in several fruits, including citrus fruits, bananas, apples, and others [30–33].

Comparing the local (Saudi) tomato cultivars to the imported tomatoes (non-Saudi) revealed significantly higher amounts of carotenoids in the tomato skin of Saudi cultivars compared to the non-Saudi varieties (Figure 7). This variation can be caused by several factors, including climatic and environmental conditions, as previously noticed with local and imported bitter melon [34]. Temperature and light have been shown to significantly affect the content of phenolic and carotenoid compounds in the tomato fruit [35]. The majority of tomato varieties cultivated in Saudi Arabia are exposed to higher temperatures and light compared to most imported tomatoes grown in colder countries, which might be the main reason for the higher amounts of antioxidants in local cultivars. Genotype can be another important factor

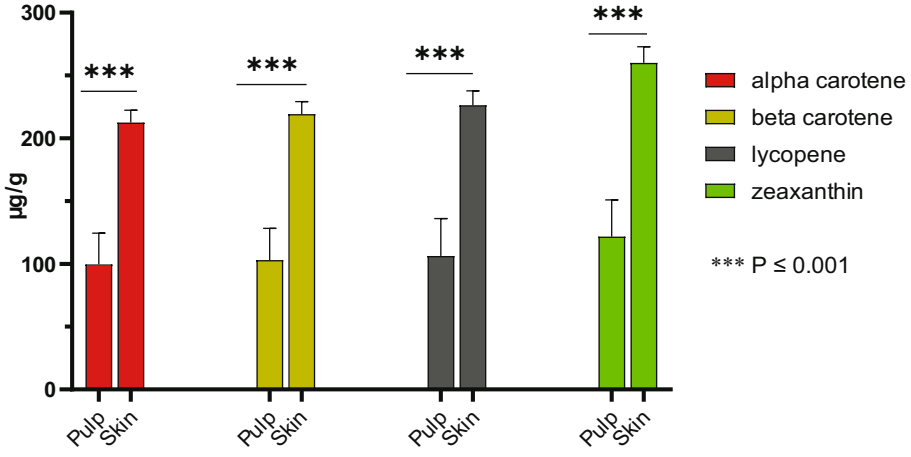

Figure 6: Carotenoid concentrations in the pulp and skin of tomatoes, compared using an independent samples *t*-test. The data represent the mean ± standard error mean.

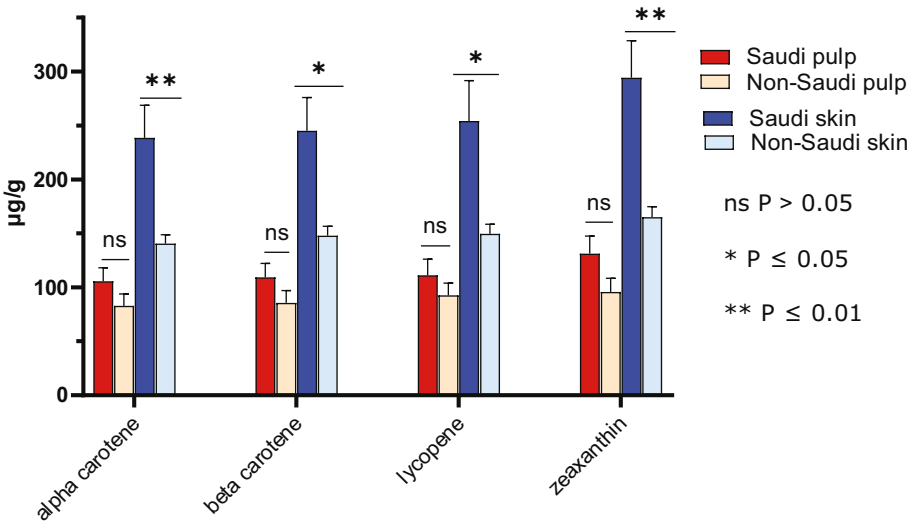

Figure 7: Carotenoid concentrations in the Saudi and non-Saudi tomatoes, compared using an independent samples *t*-test. The data represents the mean ± standard error mean.

behind the variation of the antioxidants among tomato varieties, as previously shown in Spanish traditional tomatoes [14].

The independent samples *t*-test revealed no significant difference between the Ramis tomato and the other local tomato varieties ($P > 0.05$). Nevertheless, a considerable variation was observed among the cultivars in each group of tomatoes. For example, the Kharj tomato in the non-Ramis cultivars contained the highest content of carotenoids, while the Tabuk tomato contained the highest amounts of phenolics. Therefore, one cultivar can have a high content of one class of antioxidants but lower amounts of another important class of antioxidants. Moreover, the cultivation location and agronomic conditions might lead to significant variations in the antioxidant content of the same cultivar. Among the Ramis samples, the Ramis tomato cultivated in its original place contained the highest phenolic content, whereas the Ramis tomato cultivated in Awjam village contained the highest content of carotenoids as well as high quantities of phenolics. The cultivation of Ramis tomatoes in Jarudiyah village had a detrimental effect on the carotenoid content, while the phenolic content remained unaffected.

5 Conclusion

A simple and efficient one-step extraction method employing a methanol and petroleum ether mixture was developed in this study to simultaneously extract two important antioxidant classes, phenolics and carotenoids, from tomato varieties. The developed extraction process reduced the need for evaporation, re-solubilisation, and homogenisation steps. Tomato cultivars differed significantly in their concentrations of phenolic and carotenoid antioxidant compounds. Ramis Awjam tomatoes grown in Saudi Arabia contained high concentrations of both antioxidant components, which may be attributed to ecological factors such as higher temperatures and light exposure in Saudi Arabia. The tomato skin portion had higher phenolic and carotenoid levels than the pulp. The abundance of antioxidant substances in the tomato skin emphasises the importance of this wasted part, which is typically removed, resulting in a significant loss of antioxidant components. It is worth mentioning that this study is limited to developing a rapid and straightforward method for extracting and quantifying antioxidants in tomato varieties. Further studies are recommended to evaluate the variation in biological activity and its correlation to the antioxidant content.

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(Mohammed Almasbah); resources, M.A. (Mohammed Aldholmi) and M.A. (Mohammed Almasbah); data curation, M.A. (Mohammed Aldholmi) and R.A.; writing – original draft preparation, M.A. (Mohammed Aldholmi), F.A., E.A., and R.A.; writing – review and editing, M.A. (Mohammed Aldholmi), R.A., M.A. (Mohd Amir), S.H., and M.A. (Mohammed Almasbah); visualisation, M.A. (Mohammed Aldholmi), S.H., and R.A.; supervision, M.A. (Mohammed Aldholmi) and M.A. (Mohd Amir); project administration, S.H. and M.A. (Mohammed Aldholmi). All authors have read and agreed to the final version of the manuscript.

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