JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

BIOACTIVE COMPONENTS AND ANTIBACTERIAL ACTIVITY OF RAW AND BOILED EGYPTIAN PEPPER

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https://doi.org/10.55251/jmbfs.11878

ARTICLE INFO

Received 14. 6. 2024
Revised 30. 9. 2024
Accepted 1. 10. 2024
Published xx.xx.201x

Regular article

OPEN ACCESS

ABSTRACT

In the current study, two cultivars of pepper (*Capsicum annuum* and *Capsicum frutescens*) at two maturity stages (green and red) were evaluated for their contents of some bioactive compounds and antibacterial activities for their ethanolic and aqueous extracts in both raw and heat-treated forms (boiling). Boiling treatment was performed under the Egyptian household conditions. Proximate analyses of the tested samples were determined, and the resulted data showed that ash and crude protein were declined after boiling treatment, while crude fat and total carbohydrate increased. Vitamin C, β-carotene and vitamin E as well as capsaicin contents were also estimated by HPLC, and the obtained data showed that all of those components were lowered by boiling treatment. Antioxidant activity of fresh and heat-treated pepper samples was carried out by using of 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS•) assay. Data showed that all pepper samples had high antioxidant activities which were increased because of boiling treatment. Finally, the antibacterial activity of all ethanolic and aqueous extracts as well as capsaicin standard was tested against both Gram-positive and Gram-negative pathogenic bacteria. Only, ethanolic extracts partially inhibited all the tested organisms except, *Bacillus cereus* which was completely inhibited by both ethanolic and aqueous extracts.

Keywords: Sweet pepper, Chilli pepper, Capsicum, Boiling, Bioactive compounds, Antioxidant activity, Antibacterial activity

INTRODUCTION

The genus *Capsicum*, part of the nightshade family (*Solanaceae*), includes plants known for their edible fruits. These fruits can be eaten fresh, cooked, dried into powder, used in sauces, or made into oleoresin. Though native to the Americas, *Capsicum* plants are now grown all over the world. Among them, five species have been domesticated: *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*, along with roughly 20 recognized wild species (**Berke & Shieh, 2001**).

The species most commonly used in food processing is Capsicum annuum, widely recognized as pepper. Peppers are typically classified into three categories based on their flavor and shape: bell peppers, sweet peppers, and hot peppers. They are incorporated into foods for their roles as colorants, flavor enhancers, or for their spiciness. However, there is ongoing debate about the correct terminology for Capsicum fruits whether whole or in powdered form, sweet or spicy—as their names differ depending on the country, language, and local practices (**Santos *et al.*, 2012**). Peppers contain phenolics and flavonoids (**Bae *et al.*, 2012**), carotenoids (**Ha *et al.*, 2007**), vitamin C, vitamin E (**Garcıa-Closas *et al.*, 2004**) and alkaloids (**Srinivas *et al.*, 2009**), which play important roles in human health. In other studies, antioxidant activities in peppers were measured by radical-scavenging activity (**Conforti *et al.*, 2007; Ademiluyi *et al.*, 2011**), inhibition of lipid peroxidation (**Menichini *et al.*, 2009**) and metal-chelating activity (**Ciz *et al.*, 2010**). Capsaicinoids and carotenoids exhibit anticancer (**Aggarwal *et al.*, 2008; Hwang *et al.*, 2009**), and antioxidant activities (**Matsufuji *et al.*, 1998; Anandakumar *et al.*, 2008; Johnson, 2009**). Flavonoids have been shown to act as antioxidants, and they possess anti-inflammatory (**Loke *et al.*, 2008**), antiallergic (**Seelinger *et al.*, 2008**), and antibacterial activities (**Hong *et al.*, 2006**). The antioxidant activity of pepper extracts involves bioactive compounds, such as polyphenols, carotenoids, capsaicinoids and ascorbic acid (**Alvarez-Parrilla *et al.*, 2010; Hervert-Hernández *et al.*, 2010; Jeong *et al.*, 2011**).

Chili peppers are widely used in foods around the world for their spicy flavor, distinctive aroma, and ability to delay spoilage. With capsaicin levels ranging from mild to extremely hot on the Scoville scale, they offer diverse options for consumers globally. Beyond their culinary applications, chili peppers have been investigated for their antimicrobial and antifungal effects. This has led to ongoing research into the potential use of chili pepper extracts as natural alternatives to synthetic preservatives in the food industry. As antibiotic-resistant foodborne pathogens continue to emerge, the identification of natural antimicrobials in chili peppers could prove to be a valuable asset for food scientists (**Omolo *et al.*, 2014**). The objective of this study was to assess the proximate composition, antioxidant activity, and bioactive compounds—including organic acids, vitamin C, β-carotene, vitamin E, and capsaicin—in both fresh and boiled pepper samples at two ripening stages (green and red). Additionally, the study aimed to evaluate the in-vitro antibacterial effects of capsaicin standards and extracts from both pepper samples against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Plant materials and boiling treatment

Fruits from hot chili pepper (*Capsicum frutescens* var. *sina*) in both immature (green) and mature (red) stages, along with sweet pepper (*Capsicum annuum* var. *goduion*) in green and red stages, were collected from the Vegetable Breeding Research Department at the Horticultural Research Institute, Agricultural Research Center, between September and December 2012. The fruits were identified at the same institute. Fresh fruits were rinsed with tap water, seeds were removed, and the edible parts were chopped into small pieces for extraction and analysis. The fruits were boiled in a covered pan with water at 100°C for 15 minutes, using an approximate ratio of 1 kg of pepper to 1 L of water. After boiling, the samples were drained using a wire mesh strainer, and the weight of the boiled samples along with the volume of the boiling water were recorded. The boiled samples were then

extracted separately with ethanol and distilled water to create ethanolic and aqueous extracts, respectively. The boiling water was filtered using Whatman paper No. 1 and subsequently used to assess all bioactive components, as well as antioxidant and antibacterial activities.

Chemicals

All chemical materials, including solvents and mineral salts, were sourced from El Gomhoryia, El Allamyia, El Nasr, and Middle East Pharmaceutical Chemical companies in Egypt. The solvents were purified prior to use. Additionally, chemicals, solvents, and standard materials employed for fractionation and identification via HPLC were obtained from Sigma/Aldrich Chemical Company in the USA.

Microorganisms and media

The Gram-positive bacteria used in this study included *Staphylococcus aureus* (DSM-20231), *Bacillus cereus* (ATCC-33018), *Micrococcus luteus* (ATCC-56814), and *Listeria monocytogenes* (ATCC-94687). The Gram-negative bacteria included *Salmonella typhimurium* (ATCC-17426), *Escherichia coli* (ATCC-69337), *Shigella* (ATCC-41789), and *Proteus vulgaris* (ATCC-13315). All bacterial strains were obtained from the Food Technology Research Institute, Agricultural Research Center, and were cultured on nutrient broth agar medium at 37°C in an Incucell incubator for 48 hours.

Proximate analyses

Proximate analyses, including moisture, ash, crude protein, crude fat, and total carbohydrates, were determined using standardized methods (**A.O.A.C, 2010).**

Extraction

Ethanolic extracts

Dried pepper samples (5 g) were extracted with 100 ml of 80% ethanol at a ratio of 1:20, while fresh and boiled pepper samples (10 g) were extracted with 100 ml of 80% ethanol at a ratio of 1:10. The extracts were processed using an ultrasonic instrument (BANDELIN SONOREX SUPER RK 514H) for 30 minutes and then left at room temperature for up to 24 hours. After this period, the extracts were filtered through Whatman paper No. 1. The resulting extracts were utilized to evaluate in-vitro antibacterial activity.

Aqueous extracts

Dried pepper samples (10 g) were extracted with 100 ml of distilled water at a ratio of 1:10, while fresh and boiled pepper samples (20 g) were extracted with 100 ml of distilled water at a ratio of 1:5. The extracts were subjected to ultrasonic processing for 30 minutes and then left at room temperature for up to 24 hours. Afterward, the extracts were filtered through Whatman paper No. 1. The resulting extracts were used to assess in-vitro antibacterial activity.

Total antioxidant capacity (ABTS•)

The total antioxidant capacity assay was conducted using the improved ABTS• method, following the protocol established by **Re *et al.* (1999)**. The percentage of inhibition was calculated using the following formula:

[A control – A extract / A control] x 100

HPLC analysis of organic acids

Organic acid fractionation was performed using the method described by **Wodecki *et al.* (1991),** employing an Agilent HPLC (Series 1200) system. This system was equipped with an autosampler injector, a solvent degasser, a UV detector set to 210 nm, and a quaternary HP pump (Series 1090). The temperature of the column (OA-1000, S/N: 5927915) was maintained at 55°C. Gradient separation was achieved using methanol and ethanol as the mobile phase.

HPLC analysis of vitamin C

Vitamin C was determined following the method outlined by **Romeu-Nadal *et al.* (2006).** One gram of dried pepper samples was mixed with a 0.3% metaphosphoric acid solution and centrifuged at 10,000 rpm for 10 minutes. The supernatant was then filtered through a 0.2 µm Millipore membrane filter, and 1-3 ml of the filtrate was collected in a vial for injection into an Agilent HPLC (Series 1200) system. This system was equipped with an autosampler injector, solvent degasser, UV detector set to 254 nm, and a quaternary HP pump (Series 3365). The column used was an Agilent 5HC-C18 (2) 250 x 4.6 mm, maintained at a temperature of 25°C. Ascorbic acid was identified by comparing the retention time of the sample peak with that of the ascorbic acid standard at 254 nm.

HPLC analysis of β-carotene

β-Carotene was determined using the method described by **Pupin *et al.*, (1999).** Dried pepper samples (5 g) were extracted with ethyl acetate (3 × 50 ml) containing butylated hydroxytoluene (BHT) at a concentration of 0.004%. The organic phase was then filtered through anhydrous sodium sulfate (50 g) and collected in an amber round-bottom flask. The dried extract was quantitatively transferred to a 10 ml volumetric flask using portions of 1.5 ml of mobile phase, which consisted of acetonitrile, methanol, and 1,2-dichloromethane in a ratio of 60:35:5 (v/v/v). The resultant solution was injected into an Agilent HPLC (Series 1200) system, equipped with an autosampler injector, solvent degasser, UV detector set to 280 nm, and a quaternary HP pump (Series 1100). The column used was an Agilent Hypersil ODS 5 µm, 4.0 x 250 mm, maintained at a temperature of 35°C.

HPLC analysis of vitamin E

Vitamin E was determined following the method outlined by **Pyka and Sliwiok (2001)**. Dried pepper samples (5 g) were extracted with hexane (3 × 50 ml) containing butylated hydroxytoluene (BHT) at a concentration of 0.004%. The organic phase was then filtered through potassium hydroxide (50 g) and collected in an amber round-bottom flask. The solution was thoroughly mixed and subsequently extracted with hexane and petroleum ether (75 and 25 ml, respectively, also containing 0.004% BHT). The combined hexane extracts were evaporated to dryness using a rotary evaporator at 40°C. The resulting extract was quantitatively transferred to a 10 ml methanol solution. A vial containing this solution was injected into an Agilent HPLC (Series 1200), which was equipped with an autosampler injector, solvent degasser, UV detector set at 290 nm, and a quaternary HP pump (Series 1100). Gradient separation was performed using methanol and water in a 9:1 (v/v) ratio as the mobile phase, with a flow rate of 1.5 ml/min. The temperature of the column (Agilent Hypersil ODS 5 µm, 4.0 x 250 mm) was maintained at 35°C. The injection volume was 20 µl of a standard vitamin E solution in ethanol.

HPLC analysis of capsaicin

Capsaicin content was determined following the method of **Collins *et al.* (1995)** using an Agilent HPLC (Series 1200) system equipped with an autosampler injector, solvent degasser, and a UV detector set to 280 nm, along with a quaternary HP pump (Series 1100). The column used was an Agilent 5HC-C18 (2) 250 x 4.6 mm, maintained at a temperature of 35°C. The mobile phase consisted of methanol and water in a ratio of 30% to 70%. A standard solution of capsaicin was prepared at 50% concentration in methanol by diluting a 2 mg stock solution. Capsaicin was identified by comparing the retention time of the sample peak with that of the capsaicin standard at 280 nm.

Agar-disc diffusion assay

Ethanolic and aqueous extracts were evaporated using a vacuum oven (NAPCO USA, Model: 5831) at 40°C until a sticky residue was obtained from both pepper extracts. The agar disc diffusion method was utilized to determine antibacterial activity, following the **NCCLS guidelines (1999)**. The residual sticky material from both the ethanolic and aqueous extracts was dissolved in ethanol and distilled water, respectively, achieving a final concentration of 10 mg/ml. Additionally, 1.5 and 10 mg of a 50% capsaicin solution were dissolved in 1 ml of ethanol to serve as standards. All samples were sterilized through filtration using a 0.45 µm Millipore express filter. For the antibacterial assay, bacterial cultures were suspended in sterile water and diluted to approximately 10^6 CFU/ml. A 100 µl aliquot of this suspension was spread onto the surface of nutrient agar using a sterile spatula. Sterile paper discs (Whatman No. 1) that had absorbed 10 µl of the pepper extracts were placed onto the agar at specific intervals and pressed gently to ensure contact. The plates were then incubated at 37°C for 48 hours. After incubation, the inhibition zones around the discs, where bacterial growth did not occur, were measured in millimeters. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (DIZ) for the tested bacteria, reported in millimeters (mm).

Statistical analysis

All results were expressed as means ± standard deviation. Data analysis was performed using the Statistical Analysis System (SAS) software package version 9.1. Significant differences between mean values were assessed using the least significant difference (LSD) test, with a significance level set at $P > 0.05$.

RESULTS AND DISCUSSION

Proximate analyses of fresh and boiled pepper samples

The proximate analyses of fresh and boiled pepper samples are summarized in Table 1. The data indicate that fresh peppers have a high moisture content, ranging from 89.25% to 94.17%, with no significant changes observed after boiling. The

ash content in fresh peppers ranged from 1.28% to 1.61%, which decreased in boiled samples to between 0.42% and 0.56%. Crude protein values for fresh peppers varied from 1.32% to 2.64%, and there was a slight decrease in boiled peppers, with values ranging from 1.07% to 2.60%. Fat content in fresh peppers ranged from 1.02% to 1.82%, while in boiled peppers, it ranged from 1.31% to 2.32%. Lastly, total carbohydrate content was observed to be between 1.90% and 4.68% in fresh peppers and from 2.52% to 5.28% in boiled samples. The results indicate that boiling increased the crude fat and total carbohydrate content, while other parameters decreased. Furthermore, all measured parameters increased with maturation, from the green to the red stage, except for moisture content. These findings are consistent with those reported by **Simonne *et al.* (1997)**, who found moisture content ranging from 92.6% to 94.0% and fat content from 0.17% to 1.1% among different cultivars of colored bell pepper ($P > 0.05$). Within the same cultivar across the color stages used in this study, moisture and fat content did not differ significantly ($P > 0.05$). It was also noted that fat content tended to increase slightly from the mature to the fully colored stage, likely due to the rise in carotenoid pigments. Protein content ranged from 0.32 g to 0.58 g per 100 g of the edible portion.

Table 1 Proximate analyses of different pepper samples

Component	Samples	SGC	SRC	GGS	GRS
Moisture	Fresh	$92.23^{b}\pm0.11$	$89.25^{c}\pm0.27$	$94.17^{a}\pm0.26$	$93.80^{a}\pm0.07$
	Boiled	$92.05^{b}\pm0.80$	$89.24^{c}\pm0.16$	$93.73^{a}\pm0.68$	$92.66^{b}\pm0.40$
Ash	Fresh	$1.28^{a}\pm0.24$	$1.60^{a}\pm0.10$	$1.43^{a}\pm0.07$	$1.61^{a}\pm0.51$
	Boiled	$0.52^{b}\pm0.03$	$0.56^{b}\pm0.06$	$0.42^{b}\pm0.01$	$0.52^{b}\pm0.02$
Crude protein	Fresh	$1.98^{b}\pm0.25$	$2.64^{a}\pm0.22$	$1.32^{c}\pm0.11$	$2.45^{a}\pm0.24$
	Boiled	$1.79^{b}\pm0.08$	$2.60^{a}\pm0.37$	$1.07^{e}\pm0.15$	$1.40^{c}\pm0.14$
Crude fat	Fresh	$1.02^{e}\pm0.53$	$1.82^{b}\pm0.15$	$1.13^{e}\pm0.50$	$1.38^{c}\pm0.85$
	Boiled	$1.31^{c}\pm0.37$	$2.32^{a}\pm0.64$	$1.44^{bc}\pm0.46$	$1.76^{b}\pm0.57$
Total carbohydrate	Fresh	$3.34^{bc}\pm0.65$	$4.68^{a}\pm0.80$	$2.39^{cd}\pm0.73$	$1.90^{d}\pm0.70$
	Boiled	$4.15^{ab}\pm0.11$	$5.28^{a}\pm0.43$	$2.90^{cd}\pm0.20$	$2.52^{cd}\pm0.27$

Each value is expressed as the mean ± standard deviation. Mean values with different letters within a specific row indicate significant differences ($P < 0.05$). The abbreviations used are as follows: SGC = Sina Green Chilli, SRC = Sina Red Chilli, GGS = Godiuon Green Sweet, GRS = Godiuon Red Sweet.

Antioxidant activity

The free radical scavenging activity measured using the ABTS• assay demonstrated an inhibition percentage ranging from 80.13% to 87.89% in fresh pepper samples and from 90.90% to 93.62% in boiled samples, with immature peppers exhibiting higher values (see Figure 1). Boiling treatment significantly enhanced the antioxidant activities of fresh peppers. In contrast, the inhibition percentage for the aqueous extracts of fresh peppers on the ABTS radical varied between 63.87% and 79.52%. Following boiling, these values increased, indicating that the boiled pepper tissues exhibited greater antioxidant activity than the boiling water itself.

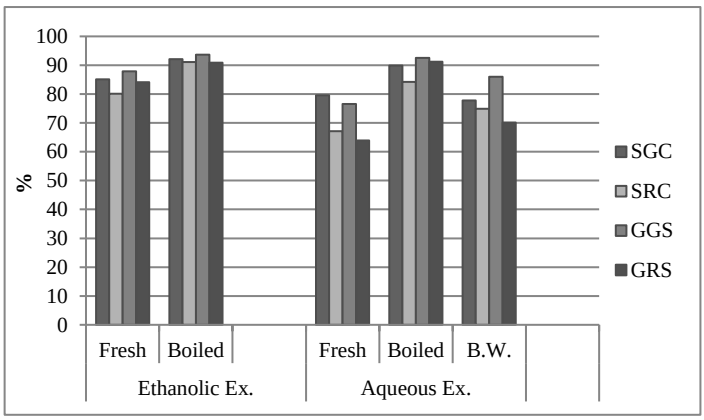

Figure 1 Antioxidant activity of both ethanolic and aqueous extracts of fresh and boiled pepper samples

Organic acid contents

Table (2) indicates that all pepper samples contain a diverse array of organic acids, with lactic, citric, malic, and fumaric acids being the most prevalent across different samples. In contrast, oxalic acid was exclusively found in the fresh Sina Green Chilli (SGC) sample, while butyric acid was detected only in the boiling water of the Godiuon Red Sweet (GRS) sample. Maleic acid appeared in fresh samples of both SGC and Godiuon Green Sweet (GGS), as well as in the boiled sample of Sina Red Chilli (SRC). Additionally, propionic acid was identified solely in fresh red pepper samples (both sweet and hot chili), while maleic acid was limited to fresh green pepper samples. Notably, the concentrations of fumaric and malic acids increased as the peppers ripened from green to red, whereas citric acid levels decreased during maturation. These findings align with the study by **Serrano *et al.* (2010)**, which identified citric acid as the primary organic acid contributing to the acidity of pepper fruits, alongside other organic acids like succinic, malic, oxalic, and fumaric acids, albeit at significantly lower concentrations, typically ranging from 20 to 120 mg/100 g of fresh weight. Conversely, **Matsufuji *et al.* (2007)** reported that citric acid levels varied between 155 to 392 mg/100 g of fresh weight across different pepper samples, highlighting the variability in organic acid content based on pepper variety and ripening stage.

Table 2 Organic acids content in fresh and boiled pepper samples and the boiling water (mg/ 100g DW)

Samples		Butyric	Formic	Succinic	Propionic	Acetic	Fumaric	Malic	Lactic	Maleic	Citric	Oxalic
Fresh	SGC	ND	ND	ND	ND	ND	67.43	2.22	37.23	52.60	21.82	9.62
	SRC	ND	ND	ND	13.83	22.19	87.80	2.90	ND	ND	7.95	ND
	GGS	ND	6.41	ND	ND	9.17	14.56	0.47	ND	23.60	9.79	ND
	GRS	ND	ND	ND	21.56	8.98	66.71	2.20	ND	ND	8.77	ND
Boiled	SGC	ND	6.55	ND	1.33	ND	14.86	0.49	10.08	ND	2.01	ND
	SRC	ND	ND	9.75	ND	5.22	ND	ND	ND	9.09	2.25	ND
	GGS	ND	3.60	2.93	18.23	ND`	8.17	0.27	12.20	ND	1.25	ND
	GRS	ND	ND	16.40	ND	9.34	ND	ND	63.89	ND	2.93	ND
Boiling water	SGC	ND	ND	ND	ND	ND	ND	ND	8.81	ND	ND	ND
	SRC	ND	ND	ND	ND	ND	ND	ND	2.77	ND	0.95	ND
	GGS	ND	ND	ND	ND	ND	2.77	0.91	9.54	ND	4.12	ND
	GRS	35.33	ND	ND	ND	ND	ND	ND	184.96	ND	ND	ND

SGC= Sina Green Chilli, SRC= Sina Red Chilli, GGS= Godiuon Green Sweet, GRS= Godiuon Red Sweet
ND: Not detected

Vitamin C, β-carotene, vitamin E and capsaicin contents

Fresh peppers are among the vegetables richest in vitamin C. According to **Lee and Kader (2000),** consuming 100 grams of peppers can provide 100–200% of the Recommended Daily Allowance (RDA) for vitamin C.

Table (3) presents the vitamin C content in fresh pepper samples, which ranges from 731.61 to 790.86 mg/100 g of fresh weight (FW) and from 402.5 to 622.7 mg/100 g of dry weight (DW) in boiled samples. The levels of vitamin C in the boiling water were notably low across all samples, indicating that boiling leads to a significant reduction in vitamin C content. These findings are consistent with those of **Chuah *et al.* (2008)**, who noted substantial decreases in ascorbic acid levels after cooking peppers for just 5 minutes, with even greater reductions after 30 minutes. Similarly, Ornelas-Paz *et al*. (2013) reported a 15–87% decrease in vitamin C due to heat treatment. However, this contrasts with the findings of **Castro *et al.* (2011)** and **Ozgur *et al.* (2011),** who suggested that thermal blanching did not significantly affect the ascorbic acid content in peppers.

Table 3 Content of Vitamin C, β-Carotene, Vitamin E, and Capsaicin in Fresh, Dried, and Boiled Pepper Samples (mg/100 g Dry Weight)

Component	Samples	SGC	SRC	GGS	GRS
Vitamin C	Fresh	731.61	748.89	761.12	790.86
	Boiled	402.50	505.11	560.32	622.74
	B.W.	6.50	3.90	1.90	6.70
β- carotene	Fresh	19.600	44.650	14.190	40.050
	Boiled	5.120	30.480	5.150	29.000
	B.W.	0.001	0.004	0.001	0.003
Vitamin E	Fresh	15.1859	16.9884	20.7337	24.5232
	Boiled	5.7723	6.0411	6.5052	6.7296
	B.W.	0.0004	0.0030	0.0003	0.0002
Capsaicin	Fresh	226.42	212.84	110.68	58.56
	Boiled	60.58	39.21	14.77	7.03
	B.W.	ND	ND	ND	ND

SGC= Sina Green Chilli, SRC= Sina Red Chilli, GGS= Godiuon Green Sweet, GRS= Godiuon Green Sweet
ND: Not detected
B.W. = Boiling water resulted from boiling treatment

The findings indicate that vitamin C levels were higher in sweet peppers compared to chili peppers and increased during the maturation process. This observation aligns with the studies of **Mozafar (1994), Simonne *et al.* (1997), Howard *et al.* (2000),** and **Marín *et al.* (2004)**, all of which reported a rise in ascorbic acid levels as peppers ripen. This increase may be linked to factors such as light intensity and elevated glucose levels, which serve as precursors to ascorbic acid.

The β-carotene content is detailed in Table 3, with values ranging from 14.19 to 44.65 mg/100 g dry weight (DW) in fresh peppers, 5.12 to 30.48 mg/100 g DW in boiled peppers, and 0.001 to 0.004 mg/100 g DW in boiled water. The data reveal that red peppers possess a higher β-carotene concentration than green peppers, reflecting the increase in β-carotene as peppers mature. Additionally, the heat treatment resulted in a decrease in β-carotene levels. This finding is consistent with **Perucka and Materska (2003)**, who reported that β-carotene content in red pepper fruits ranged from 14.0 to 39.65 mg/100 g DW across various cultivars. Furthermore, boiling reduced the β-carotene content from 19.6 to 5.12, 44.65 to 30.48, 14.19 to 5.15, and 40.05 to 29.00 mg/100 g DW for green chili, red chili, green sweet, and red sweet peppers, respectively. These results corroborate the findings of **Ornelas-Paz *et al.* (2013)**, who noted a reduction of 1–45% in β-carotene content due to heat treatments.

The findings in Table 3 indicate that vitamin E levels in fresh and boiled pepper samples, as well as in boiling water, ranged from 15.19 to 24.52 mg/100 g dry weight (DW), 6.73 to 16.99 mg/100 g DW, and 0.0002 to 0.003 mg/100 g DW, respectively. The results show that heat treatment led to a noticeable reduction in vitamin E content. Additionally, sweet peppers consistently exhibited higher vitamin E levels than chili peppers across all samples. As with vitamin C, the boiling water contained low levels of vitamin E. These findings are consistent with those of **Perucka and Materska (2003)**, who reported tocopherol concentrations in red pepper fruits ranging from 36.0 to 68.3 mg/100 g DW. **Matsufuji *et al.* (2007)** also noted that α-tocopherol levels ranged from 0.49 to 5.40 mg/100 g fresh weight (FW). Furthermore, **Isabelle *et al.* (2010)** found that α-tocopherol content in *Capsicum annuum* var. grossum (green and red) was 3.06 and 24.76 µg vitamin E/g FW, respectively, while in *Capsicum annuum* var. longum (green and red chili), it was 8.50 and 56.46 µg vitamin E/g FW, respectively.

The capsaicin (CAP) levels in the samples analyzed are presented in Table 3, revealing a range of 58.56 to 226.42 mg/100 g dry weight (DW) in fresh peppers. The data indicate that capsaicin concentration is greater in chili peppers compared to sweet peppers, with green peppers exhibiting higher levels than red peppers. Boiling treatment resulted in a decrease in capsaicin content for both sweet and chili peppers. These findings are consistent with the results reported by **Sanatombi and Sharma (2008)** and **Thapa *et al.* (2009).** However, the observed capsaicin levels are higher than those documented by **Pino *et al.* (2007)** and **Orellana-Escobedo *et al.* (2012).** Additionally, **Tundis *et al.* (2013)** noted that the concentrations of the two capsaicinoids, capsaicin and dihydrocapsaicin, tend to increase as the peppers mature.

Antibacterial activity of fresh and heat-treated pepper samples

The findings presented in Table 4 demonstrate that all tested microorganisms exhibited significant sensitivity to the ethanolic extracts, with inhibition zones ranging from 5.0 to 20.0 mm against Gram-positive bacteria and 8.5 to 19.0 mm against Gram-negative bacteria. Bacillus cereus emerged as the most sensitive microorganism to both the ethanolic and aqueous extracts, followed by Shigella, Listeria monocytogenes, Salmonella typhimurium, and Proteus vulgaris, which was only sensitive to the ethanolic extracts. Generally, Gram-positive bacteria tend to be more susceptible to extracts from spices and herbs or plant essential oils than Gram-negative bacteria, as noted by **Shan *et al.* (2007).** This difference in sensitivity is believed to stem from the distinct structure of the cell envelope; antibacterial substances can easily penetrate the cell wall of Gram-positive bacteria, disrupting the cytoplasmic membrane and causing leakage or coagulation of the cytoplasm (**Kalemba and Kunicka, 2003**). Furthermore, Gram-positive bacteria are thought to be more vulnerable due to their single outer peptidoglycan layer, which acts as a less effective permeability barrier compared to Gram-negative bacteria (**Bamoniri *et al.*, 2010**).

This agrees with **Molina-Torres et al (1999), Dorantes *et al.* (2000), Zhang *et al.* (2009), Abdul Rahman *et al.* (2010).** Capsaicin demonstrated a significant inhibitory effect on all bacteria tested, producing inhibition zones that ranged from 10.0 to 15.0 mm at a concentration of 1 mg/ml, and from 12.0 to 19.0 mm at concentrations of 5 and 10 mg/ml. These results are consistent with findings from **Molina-Torres *et al.* (1999), Dorantes *et al.* (2000), Zhang *et al.* (2009), and Abdul Rahman *et al.* (2010).**

Table 4 The antibacterial effects of ethanolic extracts from both fresh and boiled peppers (at a concentration of 10 mg/ml) and varying concentrations of capsaicin (1, 5, and 10 mg/ml)

Sample No.	Sample type	Zone of inhibition (mm)*							
		Gram positive bacteria				Gram negative bacteria			
		S. aureus	*B. cereus*	*M. luteus*	*L. monocytogenes*	*E. coli*	*S. typhimurium*	*P. vulgaris*	*Shigella*
SGC	Fresh	8.0	Complete	8.0	14.0	14.0	12.0	10.0	15.0
	Boiled	9.0	Complete	6.0	12.0	10.0	9.0	10.0	11.5
SRC	Fresh	6.0	Complete	6.0	14.0	12.5	9.5	10.0	12.0
	Boiled	12.0	Complete	5.0	14.0	12.0	10.5	15.0	14.0
GGS	Fresh	8.0	Complete	9.0	20.0	13.5	15.0	10.0	18.0
	Boiled	6.0	Complete	6.0	12.0	11.0	11.0	10.0	17.0
GRS	Fresh	5.0	Complete	6.0	10.0	11.5	11.5	10.0	12.0
	Boiled	8.0	Complete	6.0	12.0	13.0	12.0	8.5	12.5
CAP 50%	1 mg/ml	11.0	Complete	13.0	13.0	13.0	15.0	15.0	10.0
	5 mg/ml	12.0	Complete	14.0	15.0	13.0	19.0	15.0	10.0
	10 mg/ml	15.0	Complete	16.0	18.0	13.0	19.0	18.0	10.0

* Mean of two disks (zone of samples – zone of control)
SGC= Sina Green Chilli, SRC= Sina Red Chilli, GGS= Godiuon Green Sweet, GRS= Godiuon Red Sweet

The results indicate that boiling treatment slightly enhanced the antibacterial activity of some ethanolic extracts from fresh peppers (see Table 4). Conversely, Table 5 reveals that all aqueous extracts were ineffective against the tested organisms, with the exception of Bacillus cereus, which was completely inhibited by these extracts. This finding aligns with **Siripongvutikorn *et al.* (2005)**, who reported that aqueous extracts of green and red chili peppers lacked antibacterial effects against the bacteria tested. Some studies have shown that capsaicin, the primary compound responsible for the pungency or heat sensation in peppers,

possesses antibacterial properties against Helicobacter pylori **(Jones *et al.*, 1997).** However, its low solubility in water (**Santamaria *et al.*, 2000**) may explain why chili extracts had no effect on the bacteria tested.

This data contrasts with findings from **Aliakbarlu *et al.* (2014)**, who observed inhibition zones of aqueous extracts of red peppers against B. cereus, L. monocytogenes, E. coli, and S. typhimurium measuring 6.7, 8.1, 7.1, and zero mm, respectively, at a concentration of 150 mg/ml. It is also possible that the concentration and/or purity of active compounds in each spice were insufficient to inhibit the tested bacteria. The antimicrobial properties of spices are influenced by several factors, including type, composition, and concentration of the spices, as well as the concentration of target microorganisms **(Fung *et al.*, 1985)**. Additional factors such as extraction methods and the forms used (e.g., essential oils versus crude extracts) can also impact results.

Moreover, research has indicated that phenolic compounds from various plant sources can inhibit different foodborne pathogens, and there is a strong correlation between total phenolic content and antibacterial activity (**Kim *et al.*, 2005; Shan *et al.*, 2007**). The antimicrobial activities of phenolic compounds may involve multiple mechanisms, including degradation of the cell wall, disruption of the cytoplasmic membrane, leakage of cellular components, alterations in fatty acid and phospholipid composition, interference with DNA and RNA synthesis, and disruption of protein translocation **(Shan *et al.*, 2007)**.

Table 5 Antibacterial Activity of Aqueous Extracts from Fresh and Boiled Peppers and Boiling Water (10 mg/ml)

Sample No.	Sample type	Zone of inhibition (mm)							
		Gram positive bacteria				Gram negative bacteria			
		S. aureus	*B. cereus*	*M. luteus*	*L. monocytogenes*	*E. coli*	*S. typhimurium*	*P. vulgaris*	*Shigella*
SGC	Fresh	NI	Complete	NI	NI	NI	NI	NI	NI
	Boiled	NI	Complete	NI	NI	NI	NI	NI	NI
	B.W.	NI	Complete	NI	NI	NI	NI	NI	NI
SRC	Fresh	NI	Complete	NI	NI	NI	NI	NI	NI
	Boiled	NI	Complete	NI	NI	NI	NI	NI	NI
	B.W.	NI	Complete	NI	NI	NI	NI	NI	NI
GGS	Fresh	NI	Complete	NI	NI	NI	NI	NI	NI
	Boiled	NI	Complete	NI	NI	NI	NI	NI	NI
	B.W.	NI	Complete	NI	NI	NI	NI	NI	NI
GRS	Fresh	NI	Complete	NI	NI	NI	NI	NI	NI
	Boiled	NI	Complete	NI	NI	NI	NI	NI	NI
	B.W.	NI	Complete	NI	NI	NI	NI	NI	NI

NI= Not inhibited, * Mean of two disks (zone of samples – zone of control), SGC= Sina Green Chilli, SRC= Sina Red Chilli, GGS= Godiuon Green Sweet, GRS= Godiuon Red Sweet

CONCLUSION

In summary, fresh sweet and chili peppers at both green and red maturity stages contain a diverse array of phytochemical components, including vitamin C, β-carotene, vitamin E, and organic acids, along with exhibiting significant antioxidant activity. While boiling these peppers in typical Egyptian household conditions enhances their antioxidant properties, it adversely affects their overall phytochemical content. Furthermore, the ethanolic extracts derived from both fresh and boiled pepper samples demonstrate potent antibacterial activity against pathogenic Gram-positive and Gram-negative bacteria responsible for foodborne illnesses, particularly Bacillus cereus. Consequently, these extracts hold potential for use in the food industry as natural preservatives to prevent spoilage and in the medical field for treating infections caused by these bacteria.

Acknowledgements: This research was full funded by the Functional Food and Nutrition department, Food Technology Research Institute, Agricultural Research Center. Authors also thank the department of Biochemistry, Faculty of Agriculture at Cairo University.

Funding: The authors did not receive support from any organization for the submitted work.

Competing interests: All the authors declare that they have no competing interest.

Data availability: Data used during the current study are available from the corresponding author.

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