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RESEARCH ARTICLE

Combined application of high pressure and ultrasound in fig paste: effect on bioactive and volatile compounds

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Abstract

The combined impact of high-hydrostatic pressure (HHP) and ultrasound (US) on the cyanidin-3-O-rutinoside (C3R), quercetin-3-O-rutinoside (Q3R), and volatile compounds from fig (*Ficus carica*) paste was investigated. The HHP increased the content of C3R and Q3R, from 70 to 133 mg/kg fw and 31 to 44 mg/kg fw, respectively. The combination of HHP and US further enhanced the extraction of these bioactive compounds. Specifically, processing fig paste with US for 5 min at 40 °C yielded approximately 250 mg of C3R/kg fw and 45 mg of Q3R/kg fw, after 20 min. More than 25 volatile compounds were identified, with benzaldehyde being the predominant compound, accounting > 75%. Trace amounts of hydroxymethylfurfural (< 0.36 mg/100 g fw) were detected in HHP-processed fig paste. The application of HHP at mild temperatures and short time, combined with US, effectively promotes the content of bioactive compounds present in fig paste without adversely affecting the fruit's volatile compounds.

Keywords Fig paste · Bioactive · Volatile compounds · High hydrostatic pressure · Ultrasound

Introduction

Fig (*Ficus carica*) is a climacteric fruit that is commonly consumed fresh, which has a characteristic flavor and aroma that determine its quality and maturity (Reyes-Avalos et al., 2016). Due to its nutritional composition, the fig is considered an important source of minerals, vitamins, dietary fiber (Barolo et al., 2014), with considerable concentration of polyphenols (Reyes-Avalos et al., 2019). The main polyphenols associated with figs are anthocyanins (Solomon et al., 2006). The anthocyanins with the highest presence reported in figs are cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside (Reyes-Avalos et al., 2019). These compounds are known to have health benefits for consumers by inhibiting inflammatory processes; as well as having anticarcinogenic and hypoglycemic properties (Castro-Acosta et al., 2016; Szymanowska et al., 2015). Further, fig contains considerable amounts of quercetin-3-O-rutinoside (Kamiloglu & Capanoglu, 2015; Reyes-Avalos et al., 2019); flavonoid able to reduce the oxidative stress (Nishimura et al., 2016), inflammatory activity (Mascaraque et al., 2014) and therapeutic effect to treat myocardial hypoxia/reoxygenation injury (Ali et al., 2021). In addition to this, it has been observed that fig

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contains benzaldehyde, a component used to inhibit the growth of carcinogenic tumors (Saitoh & Saya, 2016).

However, the fresh fig is a highly perishable (Irfan et al., 2013). So, the fig is processed in the form of dehydration or transformed into sweets and jams (Hiwale, 2015)or wine (Kadam et al., 2011) to increases its conservation; but these processes change their sensory attributes (color, flavor and texture), as well as damage their nutritional and functional properties (Kamiloglu et al., 2015). Therefore, it is necessary to investigate conservation alternatives for the fig without changes in these desirable attributes. An alternative, which has been well accepted, has been the use of non-thermal technologies, such as high hydrostatic pressure (HHP), since they minimally modify the quality of the food (San Martín et al., 2002).The HHP has demonstrated to be an efficient processing technology for the inactivation of microorganisms without affecting the bioactive, nutritional and sensory properties of foods (Huang et al., 2014; Keenan et al., 2012; Varela-Santos et al., 2012). Further, HHP have shown to facilitate the release of antioxidant compounds from food tissues, possible by generating cell rupture (Barba et al., 2017; Barba et al., 2016; Hernández-Carrión et al., 2015). In the last decade, HHP has been considered as a good alternative for the extraction of bioactive compounds from vegetal tissues (Barba et al., 2015; Barba et al., 2016).

Likewise, the extraction and obtaining of bioactive compounds, more efficiently, has led to various studies at present. Some of these methods are microwave-assisted extraction, ultrasound and supercritical fluids, among others (Ajami et al., 2022; Bimakr et al., 2017; Bimakr et al., 2019; Kia et al., 2018; Puértolas et al., 2016); which are characterized by short extraction time, reducing the consumption of solvent and energy, improving the quality of the extracts and increasing the yield (Chemat et al., 2017).

In particular, ultrasonic extraction involves the production, growth and collapse of vacuum bubbles (Azmir et al., 2013), which can cause cell wall rupture, consequently, greater release of compounds (Roselló-Soto et al., 2015). Furthermore, ultrasound is a relatively easy technique to use; versatile, flexible, and requires low investment compared to other extraction techniques mentioned.

Numerous studies on the effect of non-thermal methods on fruit and juice components, including polyphenols, have been carried out (Kubo et al., 2021; Noroozi et al., 2021; Putnik et al., 2019; Rodríguez-Rico et al., 2022; Wu et al., 2021), but investigations on the effects of HHP combined with moderate temperatures and ultrasound, on the content of anthocyanins, quercetins and aromatics of fig are not available.

Therefore, the objective of this work was to determine the combined effect of the application of HHP at mild temperatures and ultrasound on the concentration of cyanidin-3-rutinoside (C3R), quercetin-3-rutinoside (Q3R) and volatile compounds of fig (*Ficus carica*) var. Mission paste.

Materials and methods

Chemical reagents

Water, methanol, phosphoric acid, ammonium diacid phosphate and acetonitrile HPLC grade were supplied by JT Baker (Phillipsburg, NJ, USA). Divinylbenzene/Polydimethylsiloxane (Divinylbenzene/PDMS) SPME Fibers, quercetin-3-O-ruthinoside and cyanidin 3-O-ruthinoside and 4-nonanol standard grade were purchased from Sigma-Aldrich Company (St. Louis MI, USA).

Raw material

Figs (*Ficus carica* var. Mission) used in the current study were collected in Cd. Lerdo (Durango, Mexico; 25° 32′ 10″ N/103° 31′ 28″ W) at maturity stage IV following the scale previously described in Reyes-Avalos et al. (2019). Fruits free of physical damage and visual microbiological contamination, were selected for this study.

Application of high hydrostatic pressure

The figs were ground in a domestic blender (BRLY-Z00-013, Oster, Monterrey, NL, Mexico) until a uniform paste was obtained. Samples of 100 g of fig paste were vacuum packed. The processing conditions were selected based on preliminary fungal and yeast inactivation tests and the limitations of the HHP equipment (Isostatic press Flow Autoclave System, Avure Autoclave Systems, Model LCIP402260NCEP1MLN, Eri, PA, USA). The packed fig paste was divided into 2 batches: the first-one was processed at 350 MPa of HHP for 5, 10 and 20 min at 20 and 40 °C while, the second-one was treated at 45, 50 and 60 °C during 5 min under HHP (350 MPa). Ethylene glycol (5%) was used as pressure transmission media. The temperature in the pressure chamber was controlled by recirculation of water. Fig paste without application of HHP, with and without US, was heated, either 20 or 40 °C, and used as control for the batch treated at higher temperatures (45, 50 and 60 °C). Four repetitions were performed for all HHP treatments (n = 4). The HHP processed samples were stored at −18 °C until posterior analysis.

Ultrasound-assisted extraction of cyanidin-3-rutinoside and quercetin-3-rutinoside of fig

The process was carried out as described by Veberic et al. (2008) with some modifications. Approximately 10 g of fig paste from each treatment were placed in conical plastic tubes with 10 mL of methanol containing 1% DHT, and subjected to an ultrasound bath (Branson 3510, Danbury, CT, USA) at 8ºC for 1 h. Subsequently, the samples were centrifuged at 10 °C and 6000×*g* and the supernatant was separated. The residue was subjected to extraction twice with a methanol–water mixture (80:20) for 30 min, either in ultrasound bath or magnetic stirred, and the supernatant was separated by centrifugation. The supernatant was filtered through glass fiber filter and concentrated in a rotary evaporator (BUCHI R-210, Flawil, St Gallen, Switzerland), at 35 °C to a volume of 16 mL. The concentrate was completed to 25 mL with water HPLC grade and filtered through 0.25 µm filter.

One sample of fig treated with HHP was subjected to mechanical stirring at 200 rpm under the same conditions of time, temperature and solvent, as in ultrasound-assisted extraction. The concentration of bioactive compounds extracted by mechanical stirring were taken as reference.

Quantification of cyanidin-3-rutinoside and quercetin-3-rutinoside of fig

The filtered sample was injected into an Agilent Technology model 1200 liquid chromatograph (Agilent Technology, Santa Clara, CA, USA) equipped with a quaternary pump, a diode array detector, two Discovery® C18 15 cm × 4.6 mm, 5 µm columns (Supelco Analytical, Bellefonte, PA, USA) connected in series and a 20 µL loop. The mobile phase was comprised of 50 mM ammonium phosphate adjusted to pH 2.6 with phosphoric acid (A), 80:20 acetonitrile:ammonium phosphate (B), and 200 mM phosphoric acid (C). The gradient was: 100% A for5 min, 92% A and 8% B for 8 min, 14% B and 86% C for 20 min, 16.5% B and 83.5% C for 25 min, 21.5% B and 78.5% C for 35 min, 50% B and 50% C for 70 min, 100% A for 75 min. The cyanidin-3-rutinoside (C3R) and quercetin-3-rutinoside (Q3R) were monitored at 520 nm and 365 nm, respectively. The high purity standards (99%) were used for identification and quantification.

Analysis of volatile compounds of fig

Volatile compounds were analyzed by the headspace method using fibers covered with divinylbenzene/PDMS as stationary phase with a thickness of 65 µm as described (Guedes De Pinho et al., 2009; Mota et al., 2012). Approximately 4 g of fig paste were placed in 20 mL vial hermetically sealed vials containing 4 mL of saturated NaCl solution and 4-nonanol (internal standard) to obtain a final concentration of 0.625 mg/L. The samples were subjected to ultrasound for 15 min at 40 °C, and incubated at 40 °C with constant magnetic stirring (350 rpm) during 20 min. Likewise, samples of fig paste were carried out without applying ultrasound. Then, the divinylbenzene/PDMS fiber was exposed to the headspace of the vial for 30 min. The fiber was removed from the vial and analyzed by GC–MS in an Agilent 7890 GC–MS (Agilent, Palo Alto, CA, USA) equipped with a CP WAX 52 CB 30 mx 0.25 mm × 0.25 µm column (J&W Scientific, Folsom, CA, USA). Chromatograph conditions were injector temperature 250 °C and a temperature in the oven starting at 40 °C for 5 min and followed by ramp from 5 °C/min to 132 °C, 1 °C/min to 160 °C, 2 °C/min to 165 °C, 0.5 °C/min at 182 °C and a final time of 10 min (Solis-Solis et al., 2007). The transfer line to the MS detector was held at 250 °C, and the detector temperature was 250 °C. A flow rate of 1 mL/min of helium was used as carrier. The MS was used in electron impact (EI) mode, generated at 70 eV, the scan range m/z was 35–350, at 5.5 scans/s (Mayuoni-kirshinbaum et al., 2012). The compounds were identified by comparing the mass spectra of the signals of each component in the sample with the National Institute of Standards and Technology (NIST) database. The identification criterion was that the compound had a ≥ 90% probability of quality (de Sousa Galvão et al., 2011; Ferreira et al., 2009). Quantification was performed by means of a calibration curve with 4-nonanol ($R^2 \geq 0.99$).

Statistical analysis

The statistical analysis was performed by Analysis of Variance and comparison of treatment means by Fisher's Least Significant Difference using SAS 5.0 software at a significance level of 0.05.

Results and discussion

Cyanidin-3-O-rutinoside

Mission variety figs contain significant amounts of anthocyanins, particularly cyanidin-3-O-rutinoside (Reyes-Avalos et al., 2019), which distinguishes it as a potential healthy food for the consumer. The concentration of cyanidin-3-O-rutinoside (C3R) of fig paste subjected to HHP, two temperatures (20 and 40 °C) and ultrasound are shown in Fig. 1. The combined capacity of HHP application for 5 min, 40 °C and ultrasound was the most effective in the extraction of C3R (250.5 ± 11.5 mg/kg fw). This treatment provided ~ 250% more anthocyanin than the control without HHP or ultrasound. As it can be seen, times longer than 5 min do not improve the effectiveness of the extraction;

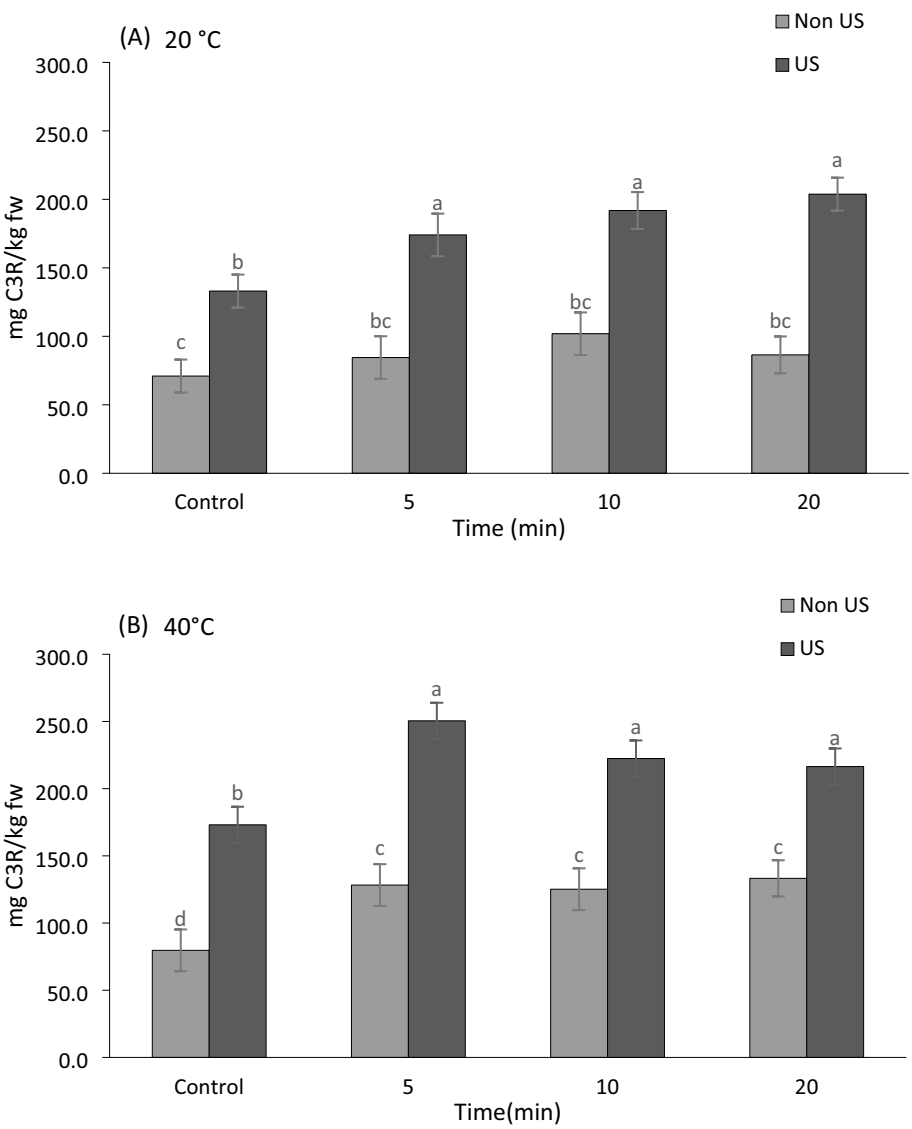

Fig. 1 Concentration of Cyanidin-3-O-rutinoside (C3R) in fig paste treated with HHP at 20 °C (**A**) and 40 °C (**B**) for different times (min), subjected to ultrasound or magnetic stirred extraction. Bars above columns represent the standard deviation (n = 4). Different letters on the columns indicate a significant difference between treatments ($p \leq 0.05$) (LSD = 36.2)

neither temperature higher than 40 °C, combined with ultrasound, increase the obtaining of C3R from the fig paste (Fig. 2).

Several authors have reported concentrations of C3R in figs, similar to those found in this study (Reyes-Avalos et al., 2019; Solomon et al., 2006). The high amounts of C3R found in the treated samples suggest that the application of relatively low HHP (350 MPa) during short times (5 min) at mild temperatures (40 °C) and ultrasound allow the release of this compound and, as a consequence, an efficient extraction from the fig paste. These results are supported by the reported by Liu et al. (2016),who found that berries treated with HHP (200 MPa) exhibited higher concentrations of anthocyanins than those not treated. Other authors have exposed that HHP could be used as a preparation for the extraction of bioactive compounds (Pérez-Rodríguez et al., 2017) and improve the functional properties of food (Vega-Gálvez et al., 2014), since, as is known, HHP can increase the solvent penetration into the cells and rupture vacuole as well as collapse and break the cell membrane (Scepankova et al., 2018; Yucel et al., 2010). In addition, it has been reported that HHP could promote the deprotonation of charged groups and break salt and hydrophobic bonds, causing changes in the proteins and their possible denaturation, consequently, breaking the cell walls and membranes of the cell and organelles (De Maria et al., 2016; Xi et al., 2011); which could imply the increase in the solubilization of compounds from the cytoplasm to the extraction solvent. This was observed in the present study, since the amount of anthocyanins present in the solvent increased considerably up to ~ 95% when ultrasound was applied after HHP. It is known that ultrasound promotes cell disruption, increasing the release of bioactive compounds (Minjares-Fuentes et al., 2014; Rodríguez-Rico et al., 2022).

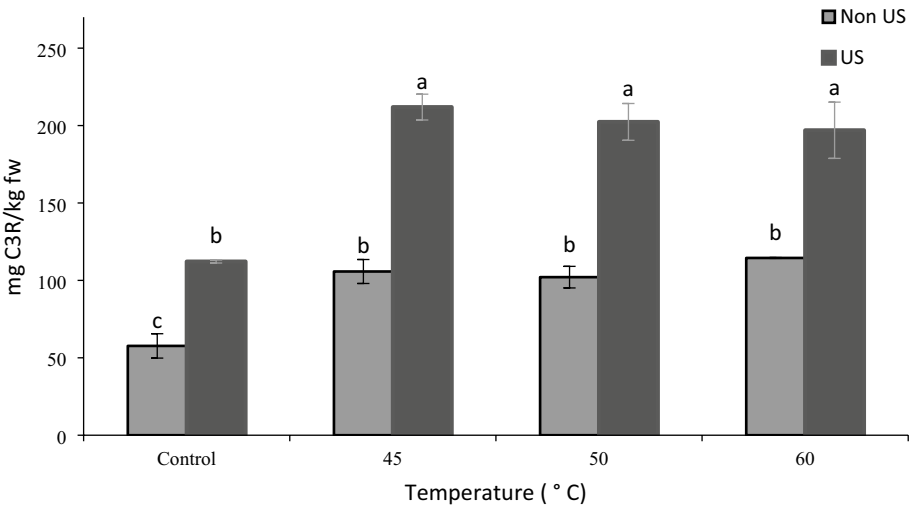

Fig. 2 Amount of C3R in fig paste treated with HHP at different temperatures for 5 min, extracted by ultrasound or magnetic stirred extraction. Bars above columns represent the standard deviation (n = 4). Different letters on the columns indicate a significant difference between treatments ($p \leq 0.05$) (LSD = 28.15)

Ultrasound increases the swelling and softening of the cell wall by hydration of the lamella compounds, which leads to the collapse of the plant tissue and the release of compounds into the environment (Minjares-Fuentes et al., 2014). Therefore, the combined application of HHP and US as an extraction procedure in fig paste, increased the obtaining of C3R, compared to only HHP or US process.

Quercetin-3-O-rutinoside

Quercetin-3-O-rutinoside (Q3R) is a flavonoid with high antioxidant capacity and anti-inflammatory and antihypertensive effects (Mascaraque et al., 2014). Q3R was the second bioactive compound with the highest concentration found in the fig paste studied. The effect of the application of HHP combined with different temperatures and ultrasound on the concentration of Q3R is shown in Fig. 3. As can be seen the amount of Q3R in the fig paste treated with HHP increased as time increased (20 min) and the temperature (40 °C), regardless sonicated or magnetic stirred extraction. Therefore, the highest extraction efficiency was observed in the treatments performed during 20 min of HHP at 40 °C (> 44 mg/kg fw). The increase in the concentration of Q3R in the samples treated with HHP at 40 °C, represented ~ 60% compared to the samples of fig paste without HHP. Likewise, the temperature above 40 °C in HHP did not increase in the amount of Q3R extracted (Fig. 4).

The high concentration of Q3R found in the samples processed with HHP at 40 °C suggests that, as in C3R, under these conditions, the solvent penetration can be increased by generating an equilibrium in the solvent concentration of the cell interior and exterior, which damages and deforms plant cells (Xi et al., 2011; Zhang et al., 2011) promoting the generation of channels, increasing the permeability of the cell membrane and increasing the solubility of intercellular compounds (Khan et al., 2019; Marcos & Mullen, 2014). Similar results were reported by Jamaludin et al. (2020) who observed that application of HHP (600 MPa) to noni fruit increased the Q3R content up to 82%.

According to the literature, the application of HHP in plant tissues, a pressure difference is developed between the inside and outside of the cell, which generates the penetration of the solvent between the damaged tissues and improves the extraction process by reducing the time and increase the amount of compounds of interest (Jun, 2013; Scepankova et al., 2018; Xi et al., 2011). Also, the use of ultrasound as an extractive procedure has widely been studied. In fact, it is known that the physical impact of US on the plant matrix can be fragmentation, erosion, capillarity, detexturation, and sonoporation or combinations thereof, which could be related to higher extraction yields (Chemat et al., 2017).

Volatile compounds

The volatile compounds identified in samples of fig Ficus carica V. mission, without HHP treatment, are shown in Table 1. Twenty-five compounds were identified which probably contribute to the aroma of the fruit. Benzaldehyde was the predominant volatile compound accounting around 77% (see Supplementary material). The concentration of benzaldehyde in the fig paste ranged from 0.6 to 1.3 mg/kg fw. These results are similar to those reported by (Oliveira et al., 2010), who found that benzaldehyde was the main volatile compound in Portuguese varieties of fig. Interestingly, benzaldehyde is known to be used in the treatment of carcinomas by inhibiting tumor promotion and/or maintenance signals (Ariyoshi-Kishino et al., 2010; Saitoh & Saya, 2016).

On the other hand, HHP treatments at different times and temperatures, with or without ultrasound, did not affect the concentration of the original volatile compounds of

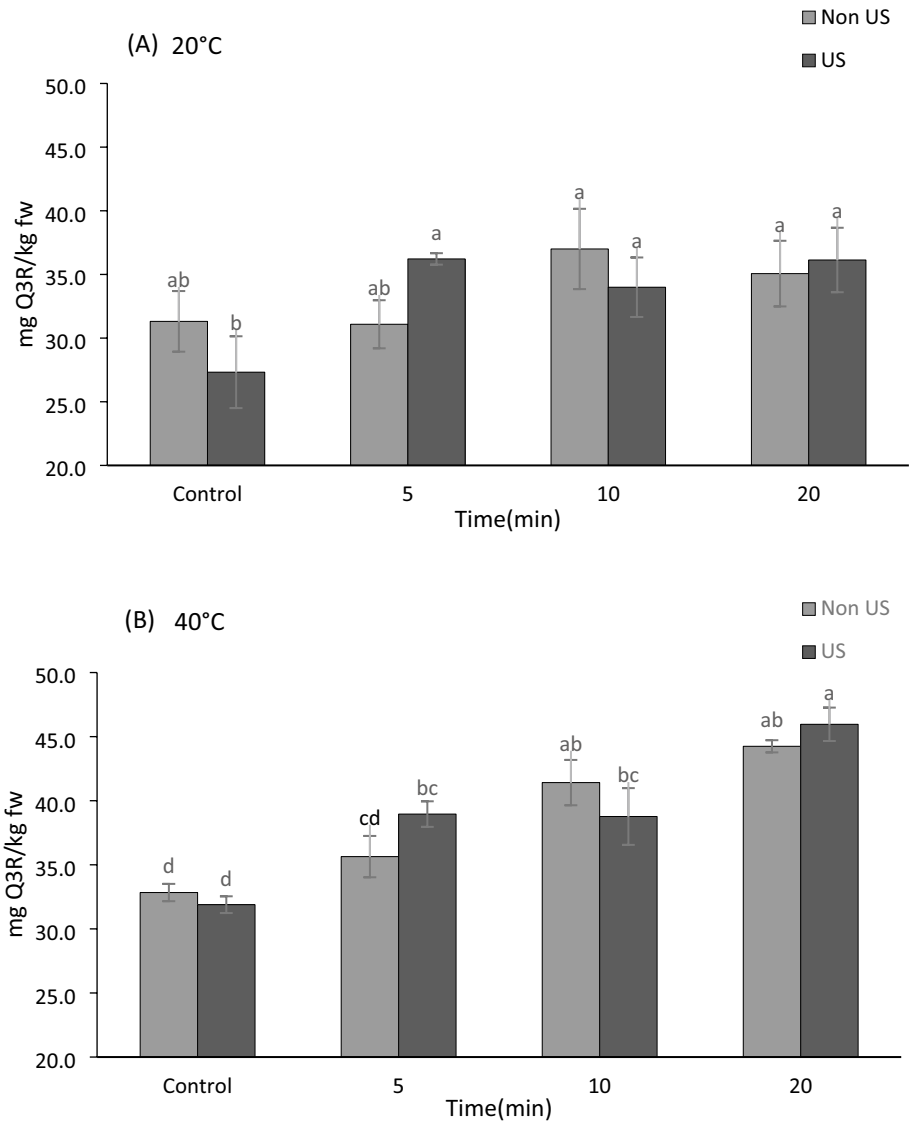

Fig. 3 Quercetin-3-O-rutinoside (Q3R) concentration in fig paste treated with HHP at 20 °C (**A**) and 40 °C (**B**) for different times (min), and a control without HHP. Bars above columns represent the standard deviation (n = 4). Different letters on the columns indicate a significant difference between treatments ($p \leq 0.05$) (LSD = 5.81)

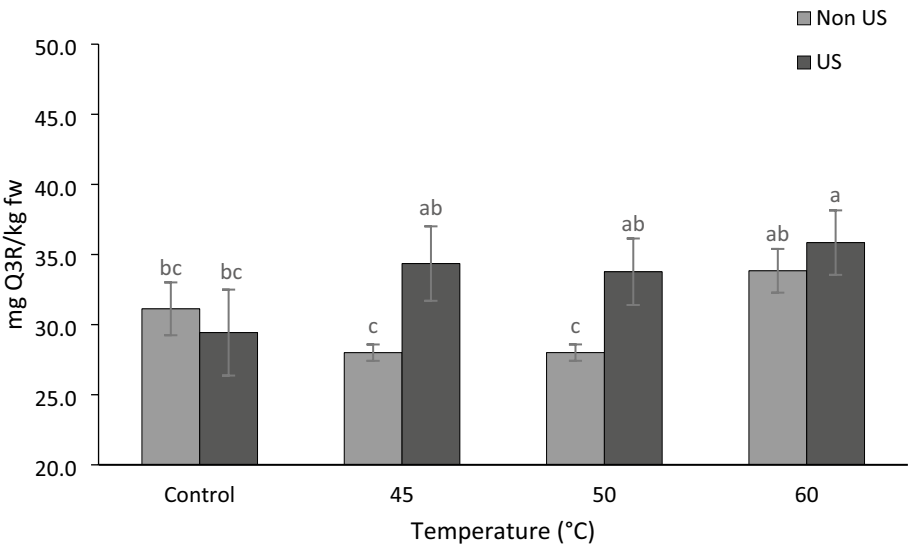

Fig. 4 Amount of Q3R in fig paste treated with HHP at different temperatures for 5 min, subjected to ultrasound or magnetic stirred extraction. Bars above columns represent the standard deviation (n = 4). Different letters on the columns indicate a significant difference between treatments ($p \leq 0.05$) (LSD = 5.75)

Table 1 Volatile compounds identified in fresh fig pulp (*Ficus carica var.* Mission) by GC–MS

Retention time (min)	Compound name	Relative Abundance (%)
22.84	non-anal	0.55
29.83	1-Octen-3-ol	1.01
30.24	2,4-Heptadienal, (E,E)-	0.85
32.03	7-Octen-2-ol, 2,6-dimethyl-	0.59
33.74	1-Hexanol, 2-ethyl-	0.94
34.86	Benzaldehyde	76.2
39.96	alpha.-Caryophyllene	0.82
40.21	Isobornylacetate	1.28
44.37	Azulene	1.90
51.12	i-Propyltricosanoate	2.25
54.28	(+)-4-Carene	0.55
54.31	3-Cyclohexene-1-methanol, alpha,alpha .4-trimethyl-	0.47
56.82	Aceticacid, phenylmethylester	1.44
58.67	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	1.24
59.47	Methylsalicylate	1.21
72.67	Benzyl Alcohol	1.37
76.67	Benzothiazole	0.59
85.85	Cinnamaldehyde, (E)-	0.54
100.89	Hexadecanoicacid, methylester	0.36
107.43	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.65
113.52	9-Octadecenoic acid, methylester, (E)-	0.76
116.02	9,12-Octadecadienoic acidmethylester	0.94
117.79	1,4,7,10,13,16-Hexaoxacyclooctadecane	0.30
119.30	9,12,15-Octadecatrienoic acid, methylester	0.76
137.86	1,4,7,10,13,16-Hexaoxacyclooctadecane	1.90

fig. However, the application of HHP for 20 min combined with 40 °C, promoted the synthesis of furan compounds in the fig paste. The furan compounds identified were furfural, 5-methyl-2-Furancarboxaldehyde, furanmethanol and hydroxymethylfurfural (HMF) (See Supplementary material), being HMF the main furan found accounting around 0.28–0.36 mg/100 g fw. However, and according to various studies, the concentration of this compound is lower than those found in food products subjected to thermal procedures such as jams (~ 1 mg/100 g fw) or dried fruits (> 100 mg/100 dw) (Shapla et al., 2018; Touati et al., 2014). The presence of furans, and especially of HMF, in fruit products subjected to HHP has been previously reported by Liu et al. (2016). They found that HHP treatments (600 MPa–1 min) in mango nectar increased the levels of HMF immediately after application and during storage. Also, it has been observed that the application of HHP (400 MPa/60 °C/1–3 h), can increase the levels of Amadori's rearrangement products, accelerating their formation and degradation (Jaeger et al., 2010), to finally their conversion to furans.

Therefore, the application of HHP at mild temperature (40 °C) during short times (5–10 min) with ultrasound assisted extraction increases the concentration of bioactive compounds extracted from the fig. Also, the presence of considerable amounts of benzaldehyde in fig paste was verified, together the presence of C3R and Q3R, could make this a food product with great functional potential for therapeutic applications. In addition, the extracts obtained from fig paste subjected to HHP for short times, mild temperatures and ultrasound can be a source of additives in the preparation of food or pharmaceutical products.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10068-023-01410-1.

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Declarations

Conflicts of interest The authors declare that they have not any conflict of interest.

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