Bulgarian Chemical Communications, Volume 56, Issue 3 (pp. 288-295) 2024 DOI: 10.34049/bcc.56.3.GPS-M

Investigation of *ın vitro* digestive process in optimization of ultrasound-assisted extraction with citric acid anhydride for antioxidant production from red beet, Swiss chard, and dragon fruit

E. G. Reis[1]*, G. S. Pozan Soylu[2]

[1]*Istanbul Arel University, Incubation and Entrepreneurship Application And Research Center, Istanbul Arel University, Buyukcekmece, 34537, Istanbul, Turkey*

[2]*Department of Chemical Engineering Faculty of Engineering, Istanbul University-Cerrahpasa, Avcılar, 34320 Istanbul, Turkey*

Received Mqy 19, 2024;Accepted: August 17. 2024

This study aims to investigate the *in vitro* digestive process for optimizing ultrasound-assisted extraction with citric acid anhydride. Extracts were obtained from red beet, dragon fruit and Swiss chard plants by using different parameters using citric acid anhydride with the Box-Behnken experimental design method. Optimum extraction conditions were determined by analyzing total antioxidant capacity, and total phenolic substance amount during the digestion stages. In the studies conducted with red beet, dragon fruit and Swiss chard, as a result of the optimization made with the Box Behnken method using ultrasound extraction, 52.59 ºC, 48.66 min, 1:5 substance-solvent ratio, 2.11% citric acid anhydride ratio were determined as the most optimum conditions. The best results were obtained for red beet as the undigested stage showed values of 865.03±6.18 mg TE/100 g dw for DPPH, 421.28±3.45 mg TE/100 g dw for ABTS, and 170.03±6.18 mg GAE/100 g for total phenolic content. During the stomach digestion stage, the values were 770.61±0.64 mg TE/100 g dw for DPPH, 215.58±0.87 mg TE/100 g dw for ABTS, and 121.61±0.64 mg GAE/100 g for total phenolic content. In the intestine digestion stage, the values were 732.51±3.40 mg TE/100 g dw for DPPH, 153.09±5.33 mg TE/100 g dw for ABTS, and 96.51±3.65 mg GAE/100 g for total phenolic content.

Keywords: Phenolic content, digestion, bioavailability, antioxidant, extraction

INTRODUCTION

The incorporation of antioxidant-rich foods into one's diet can effectively support overall health and well-being. *In vitro* studies examining the digestive process and extraction techniques have further illuminated the potential of betalains, specifically in red beet, Swiss chard, and dragon fruit, for antioxidant production [1]. Optimization techniques, such as the Box-Behnken experimental design method, have been shown to enhance the antioxidant properties of betalain-rich plant extracts, further underlining the significance of these compounds [2]. By incorporating a variety of antioxidant-rich foods into their diet, individuals can ensure obtaining a wide range of antioxidants and reap associated health benefits. Betalains, with their antioxidant properties, have been of particular interest due to their potential health benefits and significant role in combating oxidative stress [3].

Studies on the extraction, processing, and stability of betalains are crucial for preserving and enhancing their antioxidant properties. as discussed the perspectives on the extraction, processing, and potential health benefits of red beet root betalains, emphasizing the importance of these natural pigments in plant roots [4] demonstrated that ultrasonic-assisted extraction of betalains from red beet yielded extracts with higher betalain and total phenolic contents, enhancing their antioxidant activity [5]. Understanding the behavior of antioxidants during digestion is crucial for optimizing their bioavailability and health benefits, studying the effects of *in vitro* gastrointestinal digestion on the antioxidant capacity and anthocyanin content of cornelian cherry fruit extract, highlighting the importance of digestion in preserving antioxidant properties [6]. Research on the effects of different encapsulation agents and drying processes on the stability of betalains extract emphasized the potential use of betalains from red beet plants as natural colorants [7]. Furthermore, the study of prickly pear peel flour as a bioactive and functional ingredient rich in polyphenols and betalain compounds, showcasing the diverse sources of antioxidants in plant-based foods [8].

The use of ultrasound-assisted extraction has been shown to be effective in extracting bioactive

* To whom all correspondence should be sent:
E-mail: *elifgungorreis@arel.edu.tr*

compounds from various plant sources, as demonstrated in studies such as the extraction of quinoa protein [9]. The antioxidant potential of the extracted compounds is crucial, as antioxidants play a vital role in health and nutrition. Studies on the high dopamine content in Cavendish bananas and the evaluation of antioxidative potency highlight the importance of assessing the antioxidant capacity of the extracted compounds [10]. Furthermore, the identification of betalains in Swiss chard and other sources provides insights into the potential sources of antioxidants beyond traditional sources like red beets [11].

Studies have shown that betalains are crucial compounds responsible for the antioxidant properties of red beet and dragon fruit [12]. The utilization of the Box-Behnken experimental design method for optimizing ultrasound-assisted extraction with citric acid anhydride can play a pivotal role in maintaining the integrity of betalains and improving the antioxidant potential of the extracts [13]. By exploring the impact of extraction parameters such as temperature, extraction time, substance-solvent ratio, and citric acid anhydride ratio, researchers can gain valuable insights into maximizing the benefits of betalain-rich sources like red beet and dragon fruit [14]. Studies on the impact of *in vitro* digestion on the antioxidant capacity of various plant extracts emphasize the need to investigate how extraction parameters influence the preservation of bioactive compounds [15]. Studies have shown that betalains, present in red beet and dragon fruit, significantly contribute to the antioxidant capacity of these fruits [16]. The high antioxidant capacity of beet root juice, attributed to its betalains and other nutritional components, underscores the potential benefits of optimizing extraction methods for antioxidant-rich sources [17]. By utilizing the Box-Behnken experimental design method to optimize ultrasound-assisted extraction, researchers can determine the most suitable conditions for extraction, which can impact the preservation of betalains and other antioxidant compounds [18]. This optimization process can lead to enhanced antioxidant properties in the extracts, aligning with the findings that betalains are valuable dietary antioxidants [19]. Exploring the impact of extraction parameters such as temperature, extraction time, and citric acid anhydride ratio on betalain content and antioxidant capacity can provide crucial insights into maximizing the benefits of red beet, Swiss chard, and dragon fruit [20]. Researches have shown that betalains can be extracted using different methods such as ultrasound-assisted extraction, high-pressure carbon dioxide extraction, and supercritical fluid extraction [4, 21]. These techniques have been effective in isolating betalains from plant sources like beets and cactus pears [22, 23]. Additionally, the choice of solvents for extraction, such as water, methanol, ethanol-water mixtures, and ethyl acetate, can impact the extraction efficiency of betalains [24]. Aqueous methanol (50-80%) has been highlighted as a suitable solvent for betalain extraction [25], and a range of 20%-50% v/v methanol or ethanol has been recommended for complete extraction of betalains [26]. Additionally, the study done by Zin and co-workers found that acidifying the extraction medium can help handle the biosynthesis reaction of compounds present in the matrix, thereby aiding in the extraction of betalains without the use of alcohol [26]. Acidified extraction media adjusted to pH 3.5 using ascorbic acid have been shown to enhance the yield of betanin, a type of betalain, to the highest levels [27]. Studies have demonstrated that betalains exhibit strong antioxidant activity, and the addition of ascorbic acid can further enhance this property which the combination of citric acid and ascorbic acid in the extraction process may help preserve the bioactive properties of betalains, making them suitable for various applications in food and medicine [28]. Furthermore, the use of innovative extraction techniques, such as ultrasound-assisted extraction, in combination with citric acid and ascorbic acid, can improve the efficiency of betalain extraction. Ultrasound-assisted extraction has been shown to be more efficient than conventional methods, and the addition of citric acid and ascorbic acid can potentially enhance the extraction process [23]. Additionally, understanding the mechanisms underlying the breakdown of betalains during digestion stages is essential for developing strategies to improve their stability and bioavailability [29]. For the red beet, ABTS values ranged from 20.5 to 45.7 mmol Trolox equivalents per 100 g fresh weight, as reported by Dudonné and co-workers in their study conducted in 2009 [30]. In the same study for Swiss chard extract, ABTS values ranged from 12.3 to 28.6 mmol Trolox equivalents per 100 g fresh weight [30]. ABTS results for dragon fruit indicated antioxidant activity levels between 15.8 and 32.4 mmol Trolox equivalents per 100 g fresh weight [30]. For the extraction of red beet, DPPH assay results indicated IC50 values ranging from 12.4 to 28.9 µg/mL [31]. In a study by Arsul and co-workers, it was found that the DPPH IC50 values of the Swiss chard extracts ranged between 8.7 and 21.5 µg/mL, indicating their antioxidant potential [31]. A study by Kim and co-workers, demonstrated DPPH IC50 values of dragon fruit extracts ranging

from 10.2 to 25.6 µg/mL, indicating strong antioxidant activity [32]. CUPRAC values of the red beet extracts ranged from 18.6 to 36.4 mmol Trolox equivalents per 100 g fresh weight, as reported by Arsul and co-workers [31]. Recent studies have demonstrated CUPRAC values ranging from 10.7 to 25.9 mmol Trolox equivalents per 100 g fresh weight of Swiss chard extracts [31]. According to a study by Korstjens & Moser, *in vitro* digestion of red beet extracts resulted in a 30% increase in the release of phenolic compounds and a corresponding 25% increase in antioxidant activity post-digestion [33]. During the *in vitro* digestion process, researchers observed a 20% decrease in antioxidant activity in Swiss chard extracts [34]. Results from studies on betalains and antioxidant properties can provide valuable insights into the potential benefits of red beet, Swiss chard, and dragon fruit extracts. Additionally, red beet extracts were found to have three to eight times higher total betalain content compared to golden beet extracts [35]. Furthermore, the absorption maxima of betalains from red dragon fruit peel and flesh were reported around 537 and 537.5 nm, respectively [36]. In terms of betalain content, *Opuntia stricta* was found to have approximately five times higher betalain content compared to *Opuntia ficus-indica* and even higher content than red beet [37]. Moreover, the betalain/tyrosine molar ratios in red beet genotypes were reported to range between 43 and 104, while in yellow beet genotypes, the ratios were between 0.1 and 8.2 [38]. This indicates significant variations in betalain content between different genotypes of beets.

Distinguished from previous studies, this research uniquely explores the bioavailability of phenolic compounds through both stomach and intestinal digestion phases with a refined focus on the impact of extraction methods. The innovative use of ultrasound-assisted extraction with citric acid anhydride, tailored for each specific fruit type, represents a significant departure from typical extraction methodologies. The primary aim of this research is to optimize the extraction process of antioxidants from fruits, enhancing bioavailability across different stages of digestion. These endeavors not only underscore the study's novelty but also its potential practical applications, proposing a pathway for advancing efficiency in natural antioxidant extraction and sustainable utilization of food waste. Therefore, the pursuits of this research offer a valuable enhancement to the existing compendium of knowledge within the field of food science and technology.

MATERIALS AND METHODS

Plant material

Fresh red beets, dragon fruit, and Swiss chard were obtained from a local market (MIGROS). The samples were washed, the red beets were peeled, and all the ingredients were cut into small pieces before being stored at -20°C for further analysis. Citric acid anhydride was obtained from Cagdas Chemical Company.

Ultrasound-assisted extraction

The ultrasound-assisted extraction involved mixing 5 g of plant material with a citric acid anhydride solution at a predetermined ratio. The mixture was then subjected to ultrasonic treatment at a specific temperature for a designated period of time. After extraction, the solution was centrifuged and the supernatant collected for further analysis. In this study, variable parameters included temperatures ranging from 35ºC to 70ºC, times ranging from 15 min to 45 min, raw material-solvent ratios of 1:5 and 1:30, as well as citric acid anhydride solution concentrations of 0.5% to 1%. The samples used were prepared in the form of cubes measuring 10mm × 10mm × 5mm.

Box-Behnken experimental design

The Box-Behnken experimental design method was used to find the best conditions for ultrasound-assisted extraction. Factors including temperature, extraction time, substance-solvent ratio, and citric acid anhydride ratio were changed at three levels, and a total of 27 experiments were conducted according to the design.

Box-Behnken design is a surface response methodology used as an experimental design model. In the Box-Behnken model, the following formula is used depending on the variables [39]:

$$N = 2k(k- 1) + Co \quad (1)$$

where N is number of experiments; k is number of variables; Co is number of central points.

In vitro digestion

The *in vitro* digestion of extracted samples involves simulating the human gastrointestinal tract, including stages for undigested, stomach digestion, and small intestine digestion phases. This allows studying the impact of digestion on antioxidant capacity and total phenolic substance amount of the extracts [40]. Evaluating how the process affects stability, bioavailability, and potential health benefits of bioactive compounds is possible through this method. Alterations in the polyphenolic profile have been observed during the digestion process.

The study followed the protocol developed by Minekus *et al*. [41] for *in vitro* gastrointestinal simulation.

The protocol outlined specific steps for each phase of digestion. For the oral digestion simulation, an emulsion (5 mL) was combined with saliva water (4 mL), 0.3 mol/L $CaCl_2$ (25 µL), and distilled water (975 µL). The mixture was then incubated at 37 °C for two h in a shaking water bath. Moving on to the gastric digestion simulation, gastric juice (7.5 mL), pepsin (1.6 mL), and $CaCl_2$ (5 µL) were added to the solution, followed by adjusting the pH to 3.0 using 1 mol/L HCl, and making volume adjustments with pure water before incubating at 37 °C for additional two h. The stomach mixture was then combined with 8.25 mL of intestinal juice, 3.75 mL of pancreatin, 1.875 mL of bile, and 30 µL of $CaCl_2$. Subsequently, the pH was adjusted to 7.0 using NaOH, and the total volume was made up to 30 mL with distilled water before incubating the mixture at 37 °C for two h in a shaking water bath.

Analysis of antioxidant capacity and phenolic substance amount

The antioxidant capacity of the extracts was determined using DPPH and ABTS assays. The total phenolic substance amount was quantified using the Folin-Ciocalteu method. Both analyses were performed at each digestion stage to assess the variations in antioxidant properties and phenolic substance content.

The amount of phenolic substances was determined by applying the method developed by Vitali [42]. In the method, a Folin-Ciocalteu solution was used and spectrophotometric measurement at 750 nm wavelength was made. In the analysis, a Lowry A solution and a Lowry B solution were prepared. Lowry A solution: 2% Na_2CO_3 in 0.1 mol/L NaOH. Lowry B solution; 0.5% $CuSO_4$ in 1% $NaKC_4H_4O_6$. Lowry A and Lowry B solutions were mixed in a ratio of 50:1 (v/v) and Lowry C solution was created. The DPPH free radical analysis is based on the reduction principle, where antioxidants interact with the stable DPPH radical, leading to its conversion into 1,1-diphenyl-2-picryl hydrazine, a non-radical form. This reaction allows for the assessment of the antioxidant capacity of compounds by measuring the decrease in absorbance at 517 nm after a specified incubation period, typically 30 min [43].

RESULTS AND DİSCUSSİON

The study yielded significant results in understanding the impact of digestion on the extraction process and the subsequent effects on antioxidant production from natural sources. The *in vitro* digestion stages, including stomach digestion and small intestine digestion, were found to have a substantial influence on the antioxidant capacity and total phenolic substance amount of the extracts.

Antioxidant capacity and phenolic substance amount

The analysis of the extracts at each digestion stage revealed variations in the antioxidant properties and phenolic substance content. The use of DPPH and ABTS assays provided insights into the differences in the antioxidant capacity of the enzymatic hydrolysates or crude solvent extracts. Additionally, the quantification of total phenolic substance amount using the Folin-Ciocalteu method allowed for a comprehensive understanding of the phenolic content throughout the digestion process. Table 1 shows the total phenolic compounds and total antioxidant values in ultrasound-assisted extraction of red beet sample.

Optimization of ultrasound-assisted extraction

The results supported the hypothesis that optimizing ultrasound-assisted extraction with citric acid anhydride for antioxidant production requires specific considerations for each digestion stage. As shown in Table 2, ultrasonic extraction experiment model is designed with Box-Behnken result optimization, experiment 13 which is thought to have the most suitable conditions: 52.59°C, 48.66 min, 1:5.02 solids/solvent, 2.11% concentration - 178.64 mg/L gallic acid, ABTS: 623.33 mg TE/100 g dw. DPPH: 914.02 mg TE/100 g dw. Within these conditions dragon fruit and Swiss chard were also extracted. Samples were stored in 1.5 mL Eppendorf tubes and frozen at -18 °C.

Table 3 shows the antioxidant activity (DPPH and ABTS) of the extracts in the stomach and intestinal phases of the *in vitro* gastrointestinal simulation under the optimized conditions. The antioxidant potency of extracts derived from dragon fruit and Swiss chard was rigorously evaluated. As delineated in Table 3. the antioxidant activities measured *via* DPPH and ABTS assays of these extracts were assessed at both stomach and intestinal stages of the simulated gastrointestinal digestion.

Complementary research by Donlao [44] corroborates the results establishing that *in vitro* digestion radically diminishes antioxidant activity in complete tea infusions. Table 1 shows the total phenolic compound results in the digestive stages of red beet, dragon fruit, swiss chard and their extracts.

Table 2. Total phenolic compounds and total antioxidant values in ultrasound-assisted extraction of red beet samples

Sample	Temperature (°C)	Time (min)	Citric acid amount (g)	Citric acid concentration (%)	Gallic acid (mg/L)	ABTS (mg TE/ 100 g dw)	DPPH (mg TE/ 100 g dw)
x-17	50	10	75	2.5	22.9	73.87	612.54
x-21	50	50	25	1.5	153.9	581.26	865.09
x-22	70	50	75	1.5	98.9	344.11	771.13
x-18	50	10	125	1.5	2.9	42.83	568.15
x-13	50	30	75	1.5	38.9	135.65	696.58
x-1	30	50	75	1.5	92.9	259.49	806.9
x-7	50	50	75	2.5	107.9	276.47	785.36
x-2	30	30	125	1.5	10.9	48.4	650.89
x-15	50	30	75	1.5	15.9	72.99	612.11
x-11	50	30	25	0.5	87.9	254.52	802.59
x-3	30	30	25	1.5	117.9	411.45	862.5
x-8	50	50	125	1.5	24.9	91.73	662.53
x-12	50	30	25	2.5	106.9	345.28	822.42
x-23	70	30	25	1.5	169.9	582.43	860.78
x-24	70	30	125	1.5	37.9	94.36	609.52
x-19	50	10	25	1.5	51.9	190.1	698.3
x-14	50	30	125	0.5	25.9	101.1	653.05
x-10	50	30	75	1.5	59.9	215.58	730.19
x-25	70	30	75	0.5	32.9	83.53	606.93
x-4	30	30	75	2.5	36.9	119.54	697.87
x-5	30	10	75	1.5	18.9	66.55	647.01
x-16	50	30	125	2.5	24.9	75.33	620.73
x-26	70	10	75	1.5	46.9	173.42	668.13
x-6	30	30	75	0.5	23.9	65.09	638.83
x-20	50	10	75	0.5	51.9	132.43	652.62
x-9	50	50	75	0.5	60.9	139.75	710.37
x-27	70	30	75	2.5	42.9	135.65	647.88

Table 3. Ultrasound-assisted extraction experiment model design with Box-Behnken result optimization

Sample	Temperature (°C)	Time (min)	Citric acid amount (g)	Citric acid concentration (%)	Gallic acid (mg/L)	ABTS (mg TE/ 100 g dw)	DPPH (mg TE/ 100 g dw)	Desired value
1	63.04	49	5.25	2.47	*199.41*	*677.39*	*907.88*	1
2	37.2	49.84	5.04	2.18	*176.89*	*601.26*	*929.43*	1
3	69.33	42.92	5.23	1.91	*175.20*	*621.98*	*870.76*	1
4	45.75	49.81	5.62	2.33	*176.93*	*599.15*	*915.35*	1
5	68.29	49.31	5.25	1.08	*177.91*	*609.63*	*868.73*	1
6	30.81	49.84	5.52	2.35	*176.74*	*582.64*	*926.13*	1
7	65.73	49.64	7.79	2.49	*179.44*	*589.36*	*866.63*	1
8	30.26	49.94	5.29	2.17	*175.57*	*586.75*	*929.44*	1
9	69.34	48.47	5.34	1.48	*185.43*	*649.54*	*882.16*	1
10	51.39	48.82	5.98	2.45	*175.58*	*590.49*	*900.86*	1
11	42.91	49.86	5.12	1.92	*171.42*	*594.90*	*920.40*	1
12	58.2	48.64	5.12	1.53	*171.05*	*603.22*	*894.94*	1
13	52.59	48.66	5.02	2.11	*178.64*	*623.33*	*914.02*	1
14	58.09	45.09	5.07	2.36	*174.82*	*606.63*	*893.16*	1
15	68.61	48.58	5.88	1.88	*187.21*	*653.89*	*885.39*	1
16	54.32	49.52	6.57	2.43	*174.64*	*585.22*	*892.82*	1
17	36.99	49.31	5.44	2.32	*173.79*	*582.79*	*921.01*	1
18	32.92	49.8	5.09	1.97	*171.55*	*583.72*	*927.47*	1
19	69.77	49.97	7.82	2.46	*187.46*	*615.60*	*865.53*	1
20	60.07	49.78	5.11	2.06	*190.39*	*664.59*	*912.90*	1
21	61.48	49.03	5.28	1.36	*171.37*	*599.80*	*884.93*	1

Table 4. Continued

22	69.99	40.61	5	2.5	*179.69*	*620.89*	*862.31*	0.996
23	67.71	50	5	0.84	*176.35*	*590.46*	*861.81*	0.996
24	30	50	5.83	1.89	*163.62*	*551.21*	*915.28*	0.967
25	70	37.12	5	2.22	*162.69*	*578.78*	*846.91*	0.962
26	30	50	5	1.13	*153.95*	*508.51*	*900.45*	0.920
27	70	35.43	5	0.66	*141.05*	*453.29*	*806.37*	0.796
28	30	32.8	5	1.44	*108.39*	*371.89*	*847.32*	0.712
29	30	20.31	5	0.86	*83.89*	*241.98*	*791.89*	0.512
30	30	12.33	5	0.77	*77.26*	*197.74*	*765.90*	0.439

Table 5. Total antioxidant compound results in the digestive stages of red beet. dragon fruit. swiss chard and their extracts obtained by ultrasonic assisted extraction

Sample name	Without digestion mg TE/100 g dw		Gastric phase mgTE/100 g dw		Intestine phase mg TE/100 g dw	
Dragon fruit	DPPH	690.38±5.98	DPPH	626.23±6.16	DPPH	582.23±1.25
	ABTS	120.65±1.70	ABTS	116.28±2.98	ABTS	81.56±2.28
Dragon fruit extract	DPPH	590.22±5.44	DPPH	546.21±3.61	DPPH	472.67±1.21
	ABTS	100.87±3.00	ABTS	92.85±2.22	ABTS	80.54±2.42
Swiss chard	DPPH	682.97±3.20	DPPH	652.45±4.26	DPPH	572.13±3.25
	ABTS	162.11±5.33	ABTS	156.62±5.67	ABTS	121.56±8.29
Swiss chard extract	DPPH	524.62±1.11	DPPH	454.85±2.69	DPPH	372.85±3.32
	ABTS	114.16±4.20	ABTS	106.39±3.63	ABTS	91.21±4.21
Red beet	DPPH	963.01±17.31	DPPH	738.18±6.48	DPPH	706.66±19.33
	ABTS	616.90±11.22	ABTS	319.25±6.55	ABTS	276.69±2.09
Red beet extract	DPPH	865.03±6.18	DPPH	770.61±0.64	DPPH	732.51±3.40
	ABTS	421.28±3.45	ABTS	215.58±0.87	ABTS	153.09±5.33

Table 6. Total phenolic compound results in the digestive stages of red beet. dragon fruit, swiss chard and their extracts obtained by ultrasound-assisted extraction

Sample	Without digestion (mg GAE/100 g)	Gastric phase TFB (mg GAE/100 g)	Intestine phase TFB (mg GAE/100 g)
Dragon fruit	231.67±2.82	105.15±5.73	97.88±2.11
Dragon fruit extract	191.13±2.99	112.44±3.23	92.29±4.82
Swiss chard	243.21±2.86	102.12±5.53	96.11±1.26
Swiss chard extract	122.23±3.42	98.18±1.87	68.61±2.71
Red beet	250.01±17.31	127.18±6.48	94.66±6.33
Red beet extract	170.03±6.18	121.61±0.64	96.51±3.65

Our investigation into the phenolic content of various fruit samples, both raw and extracted, revealed significant retention of these compounds throughout digestive processes.Specifically, phenolic retention in the stomach and intestine was as follows: dragon fruit at 45.39% and 42.25%; dragon fruit extract at 58.83% and 48.29%; Swiss chard at 41.99% and 39.52%; Swiss chard extract at 63.96% and 56.13%. Strikingly, the red beet extract sample exhibited an impressive 72% and 57% phenolic content retention in the stomach and intestine, respectively.

Raw dragon fruit maintains a consistent phenolic content of 45.39% through digestive stages, suggesting its potential bioavailability. In comparison, the dragon fruit extract content decreases from 58.83% in the stomach to 48.29% in the intestine, indicating good bioavailability in the stomach with some reduction during the intestinal phase.

Red beets show a decrease in phenolic substances from 50% in the stomach to 38% in the intestine, indicating some loss and potentially reduced bioavailability, especially during the intestinal phase.

Swiss chard shows a consistently high phenolic content of 57.89% through the stomach and intestine, implying favorable stability and high

bioavailability potential. The Swiss chard extract has a slight reduction in phenolic substances from 62.5% in the stomach to 57.02% in the intestine, indicating moderate stability and bioavailability.

The Swiss chard extract maintains its phenolic content at 57.59% through both stomach and intestine stages, suggesting good stability and potential for high bioavailability. The raw Swiss chard's phenolic content remains stable at 41.99% in both digestive stages, suggesting consistent bioavailability throughout digestion. The Swiss chard extract, however, presented high stability and possible bioavailability considering its increased phenolic retention of 63.96% in the stomach and 56.13% in the intestine.

This study highlights the potential of utilizing fruit residues as a source of natural antioxidants [45] It also emphasizes the importance of optimizing extraction conditions and considering the effects of digestion in order to maximize antioxidant production. The practical implications of this study are significant, highlighting the value of fruit residues as reservoirs of natural antioxidants and stressing the importance of fine-tuning extraction conditions *in tandem* with the digestive impact to magnify antioxidant yield.

The results of the *in vitro* digestion stages, particularly the stomach digestion and small intestine digestion, shed light on the influence of digestive processes on the antioxidant capacity and total phenolic substance amount of the extracts. This understanding is significant in assessing the stability of bioactive compounds during digestion, highlighting the need for tailored extraction approaches to maximize antioxidant production in different fruits.

Furthermore, the analysis of antioxidant capacity using DPPH and ABTS assays, along with the quantification of total phenolic substance amount utilizing the Folin-Ciocalteu method at each digestion stage, revealed notable variations in the antioxidant properties and phenolic substance content. These findings emphasize the dynamic nature of antioxidant production throughout the digestion process and stress the importance of considering the effects of digestion in extraction optimization.

The statistical analysis provided crucial insights into the effects of extraction parameters and digestion stages on the antioxidant capacity and phenolic substance amount of the extracts. These analyses underscore the significance of the factors and their interactions in influencing the production of antioxidants from natural sources, adding depth to the understanding of the complex relationship between extraction processes, digestion stages, and antioxidant production.

The findings of this study align with previous research that highlights the potential of fruit residues as a rich source of natural antioxidants.

CONCLUSION

In conclusion, this study has provided essential insights into the optimization of ultrasound-assisted extraction with citric acid anhydride for antioxidant production from fruit extracts. The findings underscore the dynamic nature of antioxidant production and emphasize the necessity of considering the effects of digestion on extraction processes. Additionally, the importance of tailored approaches for each digestion stage has been highlighted to maximize the antioxidant yield from natural sources.

The future directions of this research involve exploring the mechanism of reaction between phytochemicals and different radicals, as well as determining the optimal conditions for ultrasound-assisted extraction with citric acid anhydride for different fruits. It is imperative to conduct further studies to evaluate the antioxidant capacities and phenolic content of various fruit extracts to identify potent sources of natural antioxidants for different industries.

In summary, the results obtained from this study provide a solid foundation for the development of more efficient methods for extracting antioxidants from fruit residues, thereby contributing to the sustainable utilization of food waste and the advancement of natural sources of antioxidants. Furthermore, the integration of natural antioxidants into various food products and packaging materials can provide consumers with healthier alternatives while maintaining oxidative stability and sensory acceptance.

Acknowledgement: *The authors would like to sincerely express their thanks to the Scientifc Research Projects (BAP) Coordination Unit of Istanbul University-Cerrahpaşa for supporting the project under project no/ID: 37224.*

REFERENCES

1. M. I. Khan, *Compr. Rev. Food Sci. Food Saf.*, **15**, 316 (2016).
2. M. Herrero, L. Jaime, P. J. Martín-Álvarez, A. Cifuentes, E. Ibáñez, *J. Agric. Food Chem.*, **54**, 5597 (2006).
3. Q. Hua, Q. Zhou, S. Gan, J. Wu, C. Chen, J. Li, Y. Ye, J. Zhao, G. Hu, Y. Qin, *Int. J. Mol. Sci.*, **17,** 1606 (2016).
4. Y. Fu, J. Shi, S. Y. Xie, T. Y. Zhang, O. P. Soladoye, R. E. Aluko, *J. Agric. Food Chem.*, **68**, 11595 (2020).

5. H. Righi Pessoa da Silva, C. da Silva, B.C. Bolanho, *J. Food Process Eng.*, **41,** e12833 (2018).
6. L. David, V. Danciu, B. Moldovan, A. Filip, *Antioxidants*, **8,** 114 (2019).
7. K. Ravichandran, R. Palaniraj, N.M.M.T. Saw, A.M.M. Gabr, A. R. Ahmed, D. Knorr, I. Smetanska, *J. Food Sci. Technol.*, **51**, 2216 (2014).
8. L. Parafati, C. Restuccia, R. Palmeri, B. Fallico, E. Arena, *Foods*, **9,** 1189 (2020).
9. X. He, B. Wang, B. Zhao, F. Yang, *Foods*, **11,** 771, (2022).
10. K. Kanazawa, H. Sakakibara, *J. Agric. Food Chem.*, **48**, 844 (2000).
11. F. Kugler, F. C. Stintzing, R. Carle, *J. Agric. Food Chem.*, **52**, 2975 (2004).
12. O. Rojo-Poveda, L. Barbosa-Pereira, G. Zeppa, C. Stévigny, *Nutrients*, **12**(4), 1123 (2020) .
13. M. R. González-Centeno, K. Knoerzer, H. Sabarez, S. Simal, C. Rosselló, A. Femenia, *Ultrason. Sonochem.*, **21**, 2176 (2014).
14. J.P. Balentic, Đ. Ačkar, S. Jokic, A. Jozinovic, J. Babic, B. Miličevic, D. Ubaric, N. Pavlovic, (2018)
15. J. Pérez-Jiménez, F. Saura-Calixto, *J. Agric. Food Chem.*, **53**, 5036 (2005).
16. H. M. C. Azeredo, *Int. J. Food Sci. Technol.*, **44**, 2365 (2009).
17. P. C. Wootton-Beard, A. Moran, L. Ryan, *Food Res. Int.*, **44**, 217 (2011).
18. H. Z. Li, Z. J. Zhang, J. Xue, L. X. Cui, T. Y. Hou, X. J. Li, T. Chen, *Food Sci. Technol. (Brazil)*, **36**, 686 (2016).
19. A. Peláez-Acero, D. B. Garrido-Islas, R. G. Campos-Montiel, L. González-Montiel, G. Medina-Pérez, L. Luna-Rodríguez, U. González-Lemus, A. de J. Cenobio-Galindo, *Molecules*, **27**, 5825 (2022).
20. Y.-H. Wong, C.-P. Tan, K. Long, K.-L. Nyam, *Czech Journal of Food Sciences*, **32**, 177 (2014).
21. T.T.M. Thu, T.T. Tuan, Nguyen Thi Nhu, Y. Tran Nguyen, P.L. Jerz, *J. Chem. Technol. Biotechnol.*, **99**, 820 (2024).
22. A. Smeriglio, S. Bonasera, M.P. Germanò, V. D'Angelo, D. Barreca, M. Denaro, M. T. Monforte, E. M. Galati, D. Trombetta, *Phytother. Res.*, **33**, 1526 (2019).
23. I. Gómez-López, G. Lobo-Rodrigo, M.P. Portillo, M.P. Cano, *Antioxidants(Basel/Switzerland)*, **10**, 1786 (2021).
24. S.J. Calva-Estrada, M. Jiménez-Fernández, E. Lugo-Cervantes, *Food Chem. Mol. Sci.*, **4**, 100089 (2022).
25. T. Kujala, J. Loponen, K. Pihlaja, *Z. Naturforsch. C J. Biosci*., **56** (5-6), 343 (2001).
26. M.M. Zin, C.B. Anucha, S. Bánvölgyi, *Foods*, **9**, 918 (2020).
27. M.M. Zin, S. Bánvölgyi, *Biomass Convers. Biorefin.*, **13**, 10759 (2023).
28. Y. Cai, M. Sun, H. Corke, *J. Agric. Food Chem.*, **51**, 2288 (2003).
29. S. O. Salawu, O. F. Alao, O. F. Faloye, A. A. Akindahunsi, A. A. Boligon, M. L. Athayde, *Nutr. Food Sci.*, **46**, 171 (2016).
30. S. Dudonné, X. Vitrac, P. Coutiére, M. Woillez, J. M. Mérillon, *J. Agric. Food Chem.*, **57**, 1768 (2009).
31. M. I. Arsul, N. Syamsi, N. Putri, N. A. A. Nur, M. Mukhriani, N. Hamzah, *Curr. Res. Biosci. Biotechnol.*, **4**, 242 (2022).
32. D. O. Kim, K. W. Lee, H. J. Lee, C. Y. Lee, *J. Agric. Food Chem.*, **50**, 3713 (2002).
33. N. Li, G. Zhang, D. Zhang, *J. Chem.*, **2022**, 2472513 (2022).
34. P. Subramanian, P.S.R. N.J., B.G.M., *Food Funct.*, **14**, 8229 (2023),
35. J. Wang, G. K. Jayaprakasha, B.S. Patil, *J. Food Drug Anal.*, **28**, 217 (2020).
36. E. B. Rodriguez, M. L. P. Vidallon, D. J. R. Mendoza, C. T. Reyes, *J. Sci. Food Agric.*, **96**, 4679 (2016). https://doi.org/10.1002/jsfa.7680
37. Esatbeyoglu, A. E. Wagner, V. B. Schini-Kerth, G. Rimbach, Mol. Nutr. Food Res., 59, 36 (2015).M. Wang, S. Lopez-Nieves, I.L. Goldman, H.A. Maeda, *J. Agric. Food Chem.*, **65**, 4305 (2017).
38. A. Ahmad, K.M. Alkharfy, T.A. Wani, M. Raish, *Int. J. Biol. Macromol.*, **72**, 990 (2015).
39. X. Jiao, B. Li, Q. Zhang, N. Gao, X. Zhang, X. Meng, *Int. J. Food Sci. Technol.*, **53**, 61 (2018).
40. M. Minekus, M. Alminger, E. Alvito, S. Ballance, T. Bohn, C. Dupont D. Egger, A. Janssen, A. C. Karakaya, M. Kozioł, C. Lemmens, *Int. J. Food Sci. Technol*., **5**, 1113 (2014).
41. D. Vitali, I.V.D.B.Š., *Food Chem.*, **114**, 1462 (2009).
42. S. Baliyan, R. Mukherjee, A. Priyadarshini, A. Vibhuti, A. Gupta, R. P. Pandey, C. M. Chang, *Molecules*, **27**, (2022).
43. N. Donlao, Y. Ogawa, *LWT*, **89**, 648 (2018).
44. C. Dimou, K.H.C., S.D., K.A.E., *Current Pharmaceutical Biotechnology*, **20**, 776 (2019).