Journal of Food Measurement and Characterization (2019) 13:1257–1268
https://doi.org/10.1007/s11694-019-00041-7

ORIGINAL PAPER

The bioactive efficiency of ultrasonic extracts from acorn leaves and green walnut husks against *Bacillus cereus*: a hybrid approach to PCA with the Taguchi method

Gokturk Ozturk[1] · Ahmet E. Yetiman[2] · Mahmut Dogan[2,3]

Received: 10 September 2018 / Accepted: 16 January 2019 / Published online: 24 January 2019

Abstract
The objective of this study was to investigate the usefulness of the hybrid approach using the Taguchi method (TM) and principal component analysis (PCA) in determining the optimum conditions for ultrasonic extracts of acorn leaves and green walnut husks, which potentially demonstrate the best bioactive efficiency against *Bacillus cereus*. First, an L_{36} ($2^1 \times 3^4$) mixed level orthogonal array design was implemented, consisting of five factors: extract type, temperature, time, solvent and concentration, respectively. Also, the total phenolic content, antiradical activity, and antibiogram analyses were investigated by design, and signal-to-noise (S/N) ratios were calculated for each trial. An analysis of variance (ANOVA) was performed using S/N ratios to estimate the effects of the measured parameters and their interactions for a single-objective TM. Following that, PCA was used to normalize the S/N ratios for each response to calculate the multi-response performance index for measuring the effects of factors on all responses. In this study, the expected optimal factor levels for each experiment were found to be different for a single-objective TM and inadequate for interpreting all responses simultaneously. The results of the study showed that the optimal conditions for all responses with PCA-based TM was found to be a 5% concentration of acorn leave extract and 50% acetone at 60 °C for 60 min, with 136.96 mg g^{-1} gallic acid equivalent, 90.75% inhibited 2,2-diphenyl-1-picrylhydrazyl and 14.33 mm inhibition zone against *B. cereus*.

Keywords Taguchi method · Principal component analysis · *Bacillus cereus* · Phenolic · Antimicrobial activity · Hybrid approach

Introduction

There have been many studies on the relationship between the intake of synthetic food additives and the development of diseases such as cancer and obesity [1, 2]. This relationship has brought about concern for the adverse effects of the synthetic food additives used in food production [3]. In addition, antimicrobial resistance has become a common problem for the whole world, and the World Health Organization has raised attention to this problem [4]. Because of these concerns, studies investigating the isolation of natural food additives have been accelerated. Many of the studies have focused on naturally occurring bioactive compounds, mainly phenolic substances from agricultural by-products with antimicrobial activity [5, 6].

It has been reported that some of the agricultural by-products rich in phenolics possess an antimicrobial effect on pathogenic microorganisms like *Bacillus cereus* [7–9]. *B. cereus* is an aerobic or facultatively anaerobic organism, a gram-positive, endospore-forming and rod-shaped bacterium that sporulates only in aerobic conditions [10, 11]. The optimum growth temperature for *B. cereus* ranges between 28 and 37 °C. However, the pathogen can grow between 10 and 50 °C, and several strains can even reproduce at 7 °C. The bacterium can multiply in pH conditions between 4.3 and 9.3 and a minimum water activity (a_w) of 0.92 [11, 12]. The bacterium is ubiquitous and may frequently spread on many kinds of foods including meat, dairy products, infant

✉ Mahmut Dogan
dogan@erciyes.edu.tr

[1] Food Technology Program, Kaman Vocational School, Ahi Evran University, 40300 Kırşehir, Turkey

[2] Food Engineering Department, Faculty of Engineering, Erciyes University, 38030 Kayseri, Turkey

[3] Tagem Food Analysis Center Co., Erciyes Teknopark Area, 38030 Kayseri, Turkey

food, spices, cereals and vegetables [10, 13]. *B. cereus* and members of its bacterial group can cause food poisoning that may arise from an intoxication (emetic syndrome) or an infection (diarrheal syndrome). An emetic syndrome occurs through the intake of a heat-stable toxin (cereulide), while a diarrheal syndrome grows from ingesting viable cells that generate enterotoxins in the small intestine [14]. The inhibition of both the microbial growth and the toxin formation of *B. cereus* is possible with the phytochemicals acquired from medicinal and non-medicinal herbal extracts [15–17].

One of the phytochemicals, phenolics, can be extracted by various techniques: either conventional techniques like soxhlet extraction, infusion and maceration, or by modern methods such as ultrasound-assisted extraction, microwave-assisted extraction, pressurized solvents, enzyme-assisted extraction and pulsed electrical fields [18]. Because there are many limitations in classical extraction techniques, which can be time-consuming, produce low yields and involve higher solvent consumption, the ultrasound extraction technique, or a combination of it with other extraction techniques, has received considerable attention in recent years. The ultrasound technique can be applied successfully in extracting phenolic compounds from plants, agricultural by-products, species, and so more yield can be achieved in a shorter time, using less solvent than conventional techniques [19].

Free radicals result from metabolic activity in aerobic systems (endogenous sources) or exogenous sources like pollution, heavy metals, tobacco and so on [20]. These radicals are deactivated by the antioxidative defense systems (enzymatic and non-enzymatic antioxidants). If there is no balance between the free radicals and the defense systems, the former is capable of causing damage to DNA, proteins, lipids and carbohydrates, thereby leading to oxidative stress, which is the most significant phenomenon in a variety of diseases [20, 21]. Phenolics can scavenge the free radicals, which act as natural antioxidants. This phenolic effect is closely associated with its antioxidant activity, depending on the phenolic type or phenolic structure–activity relationship [22]. In addition to their antioxidant activity, phenolics may exhibit antimicrobial activity against gram-positive and gram-negative bacteria [23].

Among agricultural by-products, acorn leaves (genus *Quercus*) and the green husk of walnuts (genus *Juglans*) are rich in phenolic compounds. Ferulic acid, chlorogenic acid, ellagitannins, hydrolysable tannins, gallic acid and its derivatives, and quercetin or kaempferol glycosides, have been detected in acorn leaves [24, 25]. The methanol extracts of acorn leaves (*Quercus ilex* L.) have been shown to exhibit inhibitory activity against *Brucella, Bacillus, Enterobacter, Neisseria, Pseudomonas* and *Escherichia* bacteria, and also *Candida albicans* while they do not exhibit antifungal activity [26]. Walnut and its by-products, such as leaves and green husks, contain phytochemicals with antioxidant, antimicrobial and anticarcinogenic activity [27–29]. Thirteen phenolics, including gallic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, syringic acid, syringaldehyde, *p*-coumaric acid, ferulic acid, rutin, myricetin, and juglone were identified, and juglone was found to be the major phenolic in the green walnut husk [30, 31]. As a result, it has been suggested that both acorn leaves and the green walnut husk can be used as a cheap antioxidant resource, antimicrobial agent and chemopreventive agent in both the pharmaceutical and food industry due to their phytochemical contents [26, 28, 32–34].

Using a response surface design of experiments (DoE) methodology [24, 29, 32, 35–37] along with classical and ultrasonic extraction methods, it has been reported that their some parts show antioxidant, antimicrobial and anticarcinogenic activity. The DoE consists of mathematical methods which are used to specify the relationships between the different factors that affect the process/trial and the output of the process. The DoE can be used for comparison, characterization (screening), modeling and optimization (estimation) of factors and responses [38].

One of the DoE methodologies, the Taguchi method (TM), is an extremely effective statistical approach that was developed by Dr. Genichi Taguchi, who studied the improvement of both the process and product quality of manufacturing in Japan [39]. Although the Taguchi Method has mainly been applied to various fields of industrial production, its has only been implemented in a limited number of specific case studies in the biotechnology field (e.g., food processing, fermentation, bioremediation, molecular biology and wastewater treatment) [40]. The prime objective of TM is that it can be performed with minimal experimental runs for a determination of the effects of factors on characteristic features and the determination of optimal conditions. TM has definite benefits compared to conventional statistical methodologies regarding the specification of optimal experimental conditions. The TM can specify an optimum state that has the least experimental variable conditions. The variability of the conditions is expressed by using the S/N ratio to identify control factors that minimize the variability in an experiment by decreasing the effects of noncontrollable (also called noise) factors. The maximum value of the S/N ratio corresponds to the optimal settings of an experiment that is useful for single-response cases [41, 42]. However, classical TM is not sufficient for the estimation or optimization of multi-response, problem-based studies [43]. Hence, the TM combined with PCA has been used by several authors in the engineering field to overcome the problem [43–46]. In the PCA-based Taguchi hybrid method, PCA is used to transform highly correlated independent variables into a linear combination of uncorrelated variables called principal components (PCs) that are used to select PCs whose

eigenvalue is higher than 1, and which can then be employed as a MRPI. The optimal conditions for multi-response studies can also be determined by the maximum value of MRPI that corresponds to its respective experimental run in the design matrix. The use of PCA with TM is not only to ensure objectivity but also to contribute to the development of the robustness of designs [43, 46].

As far as we are concerned, there has been no research about the estimation or optimization of the effects of ultrasonically obtained herbal extracts on microorganisms, antiradical activity, or total phenolic content (TPC) by using the PCA-TM hybrid approach. It seems that most of the published papers on TM have been interested in single response cases, and multi-response studies are not prevalent among Taguchi practitioners. Thus, the suitability of using TM with PCA for the determination of the bioactive efficiency of ultrasonic extracts of acorn (*Quercus* L.) leaves and green walnut (*Juglans regia* L.) husks against *B. cereus* was tested by considering the effects of extract type, temperature, time, solvent type and extract concentration.

Materials and methods

Samples

Acorn (*Quercus* L.) leaves and green husks of walnut (*Juglans regia* L.) were collected in Kayseri, Turkey, after harvesting them in the season of 2016. They were dried in the laboratory until 5% moisture remained [47], and then they were ground into a fine powder with a blender (Waring Blender, HGB2WTS3, Waring Commercial, USA). The powder was passed through a 0.45 µm sieve to extract a uniform particle size. The samples were stored at − 20 °C after grinding.

Chemicals

Sodium carbonate, Folin–Ciocalteu reagent, ethanol, methanol, acetone, nutrient broth and agar were purchased from Merck (Germany) while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were obtained from Fluka (Germany) and Sigma-Aldrich (Germany), respectively.

Taguchi experimental design and statistical analyses

In this study, a mixed level orthogonal array design (L_{36}: $2^1 \times 3^4$) was applied, which comprised five factors in two, three, three, three and three levels. The experimental factors and levels of the design are indicated in Table 1. For analyzing the Taguchi design, 36 experimental runs were performed, and S/N ratios were computed in compliance with a larger-the-better equation. The results of each trial were analyzed separately using the mean values. Later, data analysis was carried out to estimate the effects of the factors and their interactions by using ANOVA with a significance level of 0.05. All computations were performed using Minitab v18.1 (Minitab Ltd., Coventry, United Kingdom) statistical software.

Table 1 Factors and levels used in Taguchi mixed level array design

Factors	Levels		
	1	2	3
(A) Extract type	Acorn leaves	Walnut green husk	–
(B) Temperature (°C)	40	50	60
(C) Time (min)	30	45	60
(D) Solvent (50%)	Methanol	Ethanol	Acetone
(E) Extract concentration (%)	1.25	2.5	5

Principal component analysis (PCA)

PCA is a multivariate method that enables data to be presented in an alternative way using a smaller number of uncorrelated variables called principal components [44, 48]. By using PCA, all the response data obtained from the trials were converted into principal components (PCs) that were a linear combination of the original multi-response dataset. Before PCA, S/N ratios for each response were normalized to values between 0 and 1 by the following equation:

$$X_{iK} = \frac{X_i(K) - X_i(K)^-}{X_i(K)^+ - X_i(K)^-} \quad (1)$$

where X_{iK} represents the normalized S/N ratio of the K^{th} response data from the i^{th} experimental run, $X_i(K)$ is the S/N ratio of the K^{th} data response from the i^{th} experimental run, $X_i(K)^+$ and $X_i(K)^-$ are the maximum and minimum S/N ratios of all the experimental runs for the K^{th} data response. Afterwards, the eigenvalues and eigenvectors of the correlation matrix were calculated using the normalized S/N ratios with PCA. The PC scores were computed as follows:

$$P_i(K) = \sum_{k=1}^{m} X_{ik} \times v_k(j) \quad (2)$$

where $P_i(K)$ is the K^{th} PC equating to the i^{th} experimental run, $v_k(j)$ is the j^{th} constituent of the K^{th} eigenvector. In addition, e(K) values were calculated by dividing each eigenvalue into the sum of eigenvalues [45, 46]. MRPI values for each experimental run were calculated according to the following equation:

$$MRPI = \sum_{k=1}^{m} P_i\,(K) \times e(K) \quad (3)$$

General linear ANOVA was employed for the determination of the factors that affect the levels of each parameter. All calculations were carried out using Minitab v18.1 statistical software.

Extraction of phenolics from the samples

The extraction of phenolics from the samples was performed with respect to the Taguchi design matrix consisting of thirty-six points obtained from the Minitab software (Table 2). At first, a sonification process was conducted with an ultrasonic bath (WiseClean, WUC-A03H, 296W/1AMPS, Daihan Scientific Co. Ltd., Korea)

Table 2 L_{36} ($2^1 \times 3^4$) mixed level orthogonal array design table with factors and, experimental results that are consist of measured responses and computed S/N ratios of TPC, DPPH radical scavenging activity (RSA) and antimicrobial activity (AA) assays

Experimental run	Factors					Measured responses			Computed S/N ratios		
	A	B	C	D	E	TPC[a]	RSA[b]	AA[c]	TPC	RSA	AA
1	1	1	1	1	1	27.56	93.55	9.52	29.29106	39.39962	18.31177
2	1	2	2	2	2	55.81	92.54	10.83	34.63962	39.33631	17.77296
3	1	3	3	3	3	136.96	90.75	14.33	42.6063	39.14712	22.68712
4	1	1	1	1	1	31.04	93.10	7.36	–	–	–
5	1	2	2	2	2	52.26	92.74	6.34	–	–	–
6	1	3	3	3	3	133.11	90.54	13.01	–	–	–
7	1	1	1	2	3	109.42	90.02	13.87	40.78201	39.0872	22.84362
8	1	2	2	3	1	37.49	93.50	9.48	31.4782	39.4158	19.53617
9	1	3	3	1	2	62.43	92.26	11.46	35.90805	39.29989	21.18622
10	1	1	1	3	2	69.91	91.22	11.96	36.89028	39.20193	21.55704
11	1	2	2	1	3	120.97	88.57	15.20	41.6534	38.94551	23.63687
12	1	3	3	2	1	30.53	91.09	8.21	29.69585	39.18958	18.28334
13	1	1	2	3	1	34.41	91.18	10.48	30.73309	39.19764	20.40723
14	1	2	3	1	2	62.08	90.19	12.07	35.85865	39.10314	21.63415
15	1	3	1	2	3	117.77	88.40	13.14	41.42046	38.92891	22.37411
16	1	1	2	3	2	68.81	90.52	12.34	36.75362	39.13523	21.8263
17	1	2	3	1	3	120.69	88.17	14.19	41.63341	38.9067	23.04169
18	1	3	1	2	1	31.02	90.58	8.61	29.83367	39.14032	18.70006
19	2	1	2	1	3	19.98	77.34	7.63	26.01138	37.7685	17.6467
20	2	2	3	2	1	2.73	88.97	4.00	8.732973	38.98527	12.0412
21	2	3	1	3	2	10.64	83.98	7.98	20.53921	38.48336	18.03643
22	2	1	2	2	3	17.36	79.38	7.99	24.79024	37.9939	18.05094
23	2	2	3	3	1	3.58	88.27	4.00	11.07167	38.91623	12.0412
24	2	3	1	1	2	11.12	84.59	4.00	20.92561	38.54632	12.0412
25	2	1	3	2	1	2.62	89.14	4.00	8.36177	39.00153	12.0412
26	2	2	1	3	2	13.59	84.00	6.21	22.6628	38.48521	15.85717
27	2	3	2	1	3	20.33	78.08	8.80	26.1612	37.85043	18.88636
28	2	1	3	2	2	9.31	85.29	6.33	19.37886	38.61826	16.0235
29	2	2	1	3	3	23.44	77.09	6.96	27.39868	37.73969	16.85634
30	2	3	2	1	1	4.29	88.29	4.00	12.6547	38.91791	12.0412
31	2	1	3	3	3	21.06	77.64	7.34	26.46737	37.80198	17.31392
32	2	2	1	1	1	3.79	88.62	4.00	11.56387	38.9508	12.0412
33	2	3	2	2	2	8.71	85.59	5.35	18.80053	38.64894	14.56708
34	2	1	3	1	2	9.53	85.01	5.61	19.57876	38.58956	14.97409
35	2	2	1	2	3	18.49	79.51	8.05	25.34038	38.00866	18.11592
36	2	3	2	3	1	4.85	87.88	4.00	13.71592	38.87796	12.0412

[a]TPC: Total phenolic content as mg g^{-1} GAE

[b]RSA: DPPH radical scavenging activity (%)

[c]Diameters of inhibition zones in mm for *B. cereus*

at 50 Hz at a sample to solvent ratio of 1:10 in 50 mL glass flasks (Schott, Germany). Next, the supernatant was obtained by centrifuging (Sigma 3-30K, Germany) at 13,000 rpm for 10 min at 5 °C and lyophilized with a laboratory type freeze-dryer (Christ, Alpha 2–4 LSCplus, Germany). The lyophilized extracts were used for further analyses (Fig. 1).

Determination of TPC

The TPC of the herbal extracts was determined by using the Folin–Ciocalteu method, according to Hayta and Iscimen [49]. Briefly, 30 µL of the extract, 150 µL of 10-fold diluted Folin–Ciocalteu reagent and 120 µL of sodium carbonate (70 g L^{-1}) were placed in a 96 well polyethylene microplate. The absorbance of the mixtures were measured with a microplate-reader (Multiscan FC, Thermo-Fisher Scientific, USA) at a wavelength of 750 nm against pure water as a blank after 1 h of incubation at room temperature, and the results were calculated as mg of gallic acid equivalents, ranging from 7.81 to 125 mg kg^{-1}.

Radical scavenging activity (RSA)

Antiradical activity analysis was performed by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical in keeping with the method of Hayta and Iscimen [49]. A 30 µL volume of the extract from the microplate well was mixed with 270 µL of DPPH solution (300 µM prepared in 80% ethanol). The mixture was stirred vigorously for 5 min and allowed to stand at room temperature for 55 min in the microplate reader. At the end of the incubation period, the absorbance of the mix was measured at a wavelength of 520 nm with the microplate reader against 80% ethanol as a blank. The radical scavenging activity was expressed as the percent inhibition by using the following equation:

$$\%\ Inhibition = \left(\frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ extract}{Absorbance\ of\ the\ control}\right) \times 100$$

Antimicrobial activity (AA) assay

The strain of *Bacillus cereus* (ATCC 11778) used as a test microorganism was obtained from the Food Engineering

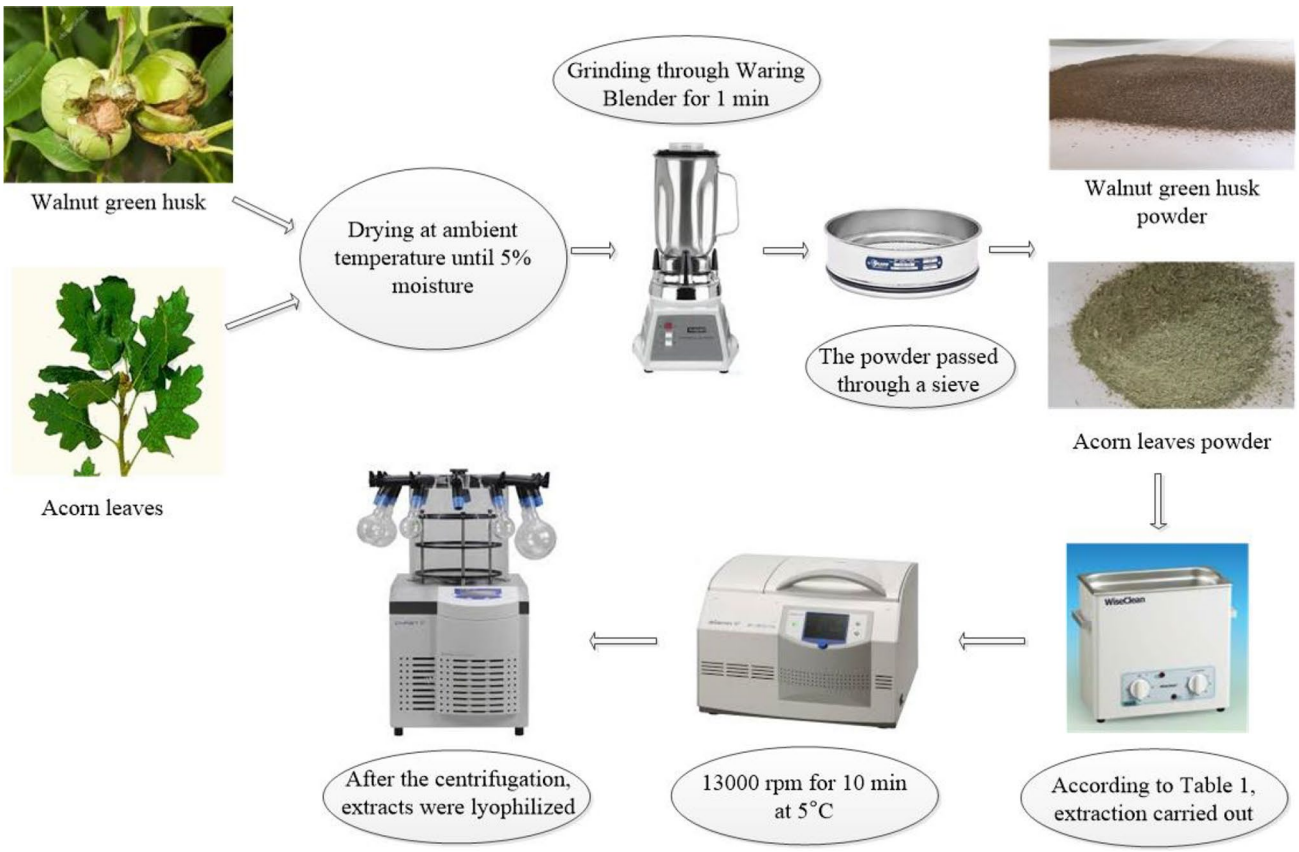

Fig. 1 Phenolic extraction methodology from acorn leaves and walnut green husk

department's culture collection of Erciyes University in Kayseri, Turkey. The determination of the antimicrobial activity of the extracts was carried out by the agar well diffusion method. The test strain was suspended in sterile nutrient broth and incubated at 35 ± 0.1 °C. The final cell concentration of the culture suspensions was adjusted by comparing it to 0.4–0.5 McFarland turbidity standards [50]. Afterward, 1 mL of the culture suspension was inoculated into 100 mL of molten nutrient agar, and approximately 15 mL of the medium was poured into 90 × 15 mm Petri dishes. After solidification of the agar plates (at 4 °C, for 1 h), four equidistant wells of 4 mm in diameter were created on the agar plates using a sterile cork borer. Fifty microliters of 1.25%, 2.5% and 5% concentrations of extracts were transferred into these wells using a sterile micropipette according to Table 2. The Petri dishes were incubated at 35 ± 0.1 °C for 18 to 24 h. After that period, the inhibition zones were measured in millimeters. All experiments were performed in triplicate [51].

Results and discussion

Results of the single objective Taguchi experiments

In this study, the aim was to estimate the most effective conditions for the maximum level of total phenolic content, radical scavenging activity and antimicrobial activity. Therefore, the usage of a larger-the-better objective function was preferred to evaluate the response variables. The mean values of the results of the response trials and their calculated S/N ratios are presented by a mixed level orthogonal array design matrix (L_{36}: $2^1 \times 3^4$) in Table 2. The numbers in each column from A to E in the design matrix indicate the environment level that implemented the factors in the experiments. The average S/N ratios with delta and rank values for each trial are also shown in Table 3. The lowest rank and highest delta value corresponded to the most effective factor for each response. Additionally, as a result of ANOVA, the degrees of freedom (DF), the sequential sum of squares (Seq SS), the adjusted sums of squares (Adj SS), the adjusted mean squares (Adj MS), and the F-value and P-value were set to interpret the factors and interactions that have statistically significant effects on each response. Tables 4 and 5 displays the ANOVA results of S/N ratios for TPC, RSA and AA, respectively. The results of the single objective TM for each experiment are discussed in separate sections below.

Table 3 Response table for means of signal-to-noise (S/N) ratios for each experiment

Factors	Level 1	Level 2	Level 3	Delta	Rank
Total phenolic content					
(A) Extract	35.95	19.12	–	16.83	1
(B) Temperature	27.19	26.55	26.57	0.64	5
(C) Time	27.88	27.04	25.39	2.49	3
(D) Solvent	27.39	25.62	27.30	1.77	4
(E) Concentration	19.74	27.45	33.11	13.38	2
DPPH radical scavenging activity					
(A) Extract	39.16	38.45	–	0.71	2
(B) Temperature	38.71	38.80	38.82	0.11	4
(C) Time	38.72	38.74	38.87	0.14	3
(D) Solvent	38.75	38.81	38.76	0.06	5
(E) Concentration	39.09	38.86	38.38	0.71	1
Diameters of inhibition zones of *B. cereus*					
(A) Extract	20.92	15.03	–	5.89	1
(B) Temperature	18.27	17.51	17.35	0.92	3
(C) Time	17.88	17.86	17.39	0.50	5
(D) Solvent	17.77	17.35	18.01	0.67	4
(E) Concentration	15.23	17.77	20.13	4.91	2

Total phenolic content

The TPC of extracts were significantly affected ($p < 0.05$) by the following factors; extract type (A), solvent (D) and concentration (E), and extract type–concentration (A*E) interaction (Table 4.). The ANOVA results for TPC demonstrated that the temperature (B) and time (C) factors, and A*B, A*C, A*D, B*D, B*E and C*D interactions, were not found to be significant ($p > 0.05$). The response table of S/N ratio means (Table 3) revealed that the extract type was the most efficient factor for TPC. Also, the following conditions: 5% concentration of acorn leaf extract, 50% acetone, 60 °C and 60 min, were found to be the most suitable for TPC that corresponded to the 3rd experimental run in Table 2.

Phenolic compounds in plants have an essential role in the formation of color and taste, and as a defence mechanism against pathogen microorganisms. Also, phenolic compounds have been reported to have a positive effect on human health; it is known that there is an inverse correlation between the consumption of a diet rich in phenols and the risk of developing disease. This relationship is due to the antioxidant properties of the phenols present in plants, thus contributing to a reduction in oxidative stress, and creating this effect at the appropriate amount of phenolics [52]. Therefore, the extraction stage is of great importance in the recovery of phenolics from agricultural by-products. Several extraction methods have been used to extract the phenolics from agro-waste. They could be influenced by the extraction parameters such as the sample type, time, temperature, solvent and its polarity. Another factor that may affect the yield and structure of phenolics is the extraction method. The ultrasound-assisted extraction method, one of the extraction techniques, has some advantages over the others. In this process, cavitation bubbles occur, causing the material used in the extraction to be destroyed, which accelerates

Table 4 Analysis of variance (ANOVA) results for S/N ratios of total phenolic content and DPPH radical scavenging activity analyses

Factors	DF	Seq SS	Adj SS	Adj MS	F	p
Total phenolic content						
A	1	2316.23	1318.18	1318.18	6738.38	0.000*
B	2	2.88	0.26	0.13	0.66	0.579
C	2	36.88	2.30	1.15	5.87	0.092
D	2	22.97	9.97	4.99	25.49	0.013*
E	2	967.04	131.36	65.68	335.75	0.000*
A*B	2	4.10	0.88	0.44	2.25	0.253
A*C	2	2.61	0.72	0.36	1.83	0.302
A*D	2	0.59	0.14	0.07	0.36	0.725
A*E	2	14.15	7.09	3.54	18.11	0.021*
B*D	4	2.16	1.73	0.43	2.21	0.270
B*E	4	3.71	2.03	0.51	2.59	0.230
C*D	4	1.22	1.22	0.30	1.56	0.373
Error	3	0.59	0.59	0.20		
Total	32	3375.12				
DPPH radical scavenging activity						
A	1	4.09699	2.77279	2.77279	228.80	0.001*
B	2	0.07813	0.00334	0.00167	0.14	0.877
C	2	0.15765	0.00285	0.00142	0.12	0.893
D	2	0.02761	0.01737	0.00868	0.72	0.557
E	2	2.74915	0.41254	0.20627	17.02	0.023*
A*B	2	0.08926	0.00270	0.00135	0.11	0.898
A*C	2	0.05099	0.00348	0.00174	0.14	0.872
A*D	2	0.06221	0.00830	0.00415	0.34	0.735
A*E	2	0.67698	0.28431	0.14216	11.73	0.038*
B*D	4	0.07579	0.07791	0.01948	1.61	0.363
B*E	4	0.01757	0.00732	0.00183	0.15	0.950
C*D	4	0.03979	0.03979	0.00995	0.82	0.589
Error	3	0.03636	0.03636	0.01212		
Total	32	8.15848				

*Significant

Table 5 Analysis of variance (ANOVA) results for S/N ratios of inhibition zone diameters of a tested strain of *Bacillus cereus*

Factors *B. cereus*	DF	Seq SS	Adj SS	Adj MS	F	p
A	1	283.424	147.901	147.901	4499.40	0.000*
B	2	5.363	2.967	1.483	45.13	0.006*
C	2	2.143	0.945	0.473	14.38	0.029*
D	2	2.344	0.194	0.097	2.96	0.195
E	2	129.372	5.256	2.628	79.95	0.002*
A*B	2	0.038	0.275	0.137	4.18	0.136
A*C	2	1.188	2.261	1.130	34.39	0.009*
A*D	2	4.392	4.133	2.066	62.86	0.004*
A*E	2	3.576	4.112	2.056	62.55	0.004*
B*D	4	6.935	2.136	0.534	16.25	0.023*
B*E	4	0.696	7.046	1.762	53.59	0.004*
C*D	4	19.625	19.625	4.906	149.25	0.001*
Error	3	0.099	0.099	0.033		
Total	32	459.195				

*Significant

mass transfer from the material into the solvent. Hence, a higher yield and higher amount of bioactive compound can be obtained compared to conventional extraction techniques [49, 53].

The total amount of phenolics in the acorn leaves in this study was higher than those found by Moreno-Jimenez et al. [34] who applied the infusion-classical technique (80 °C/10 min) to *Quercus sideroxyla, Quercus eduardii* and *Quercus durifolia* leaves, and they found that the phenolic content of the leaves varied from 15.42 to 35.15 mg of GAE/g. In a study conducted by Sanchez-Burgos et al. [54], it was determined that the TPC for different *Quercus* species leaves ranged between 6.98 and 17.26 mg of GAE/g, with the infusion method (boiling water/10 min). Similar results (7.44–35.52 mg of catechin/g) were obtained by Popovic et al. [55], but less than those reported by Rivas-Arreola et al. [56]. The ultrasound process used during the extraction process can lead to an increase in the amount of phenolics from the samples in this current study with the cavitation phenomena.

Another factor in extracting the bioactive substances from herbs is the solvent and its polarity. The effect of different solvents on the phenolic and antimicrobial activities of the herbs was investigated. As a result of these investigations, non-polar compounds dissolve in non-polar solvents, and polar ones dissolve in polar solvents, and thus the total amount of phenolics will vary depending on the type of plant used in the extraction. A 50% aqueous solution was preferred in this study since pure organic solvents extracted less phenolics than aqueous solutions [57, 58].

The highest total amount of phenolic substances in the acorn and green walnut husks was found in experiments 3 and 29, respectively. Previous studies emphasized that acorn leaves are rich in total phenolic content. The combination of acetone with water can be widely used in the extraction of tannins from plants and agro-waste. Ahmed et al. [59] investigated the effect of different solvents on phytochemical compounds and the biological activity of *Quercus dilatata*, and the aqueous acetone was found to be the best solvent system compared to single or binary solvent systems. Similar results have been reported by Alasalvar et al. [60] and Mokhtarpour et al. [61].

Tabaraki and Rastgoo [62] obtained natural antioxidants from green walnut husks by both conventional and ultrasonic extraction methods. Compared to the traditional technique, higher total phenolic content was provided by the ultrasonic method with a RSM, varying from 6.28 to 7.23 mg GA g^{-1}, with 50% ethanol as the extraction solvent. In another study, the amount of phenolics in the methanol extract from green walnut husks was found to be higher than those in petroleum ether [28]. They reported that the difference in the total amount of phenolic content in each extract could be attributed to the polarity of the solvent used in the extraction. Another reason for this condition might be attributed to the environmental conditions. Ghasemi et al. [63] stated that ecological conditions, including altitude, geography and climate affected the phytochemical compounds obtained from a methanol-based extract of green walnut husk in Iran (TPC varied from 15.15 to 108.11), which is in agreement with our results.

Antiradical activity

The analysis of variance on the DPPH radical scavenging activity (Table 4) demonstrated that the main effects of extract type (A), concentration (E) and extract type–concentration (A*E) interaction were affected by the percentage inhibition of DPPH ($p < 0.05$). In addition to this, the observed factors of temperature (B), time (C) and solvent (D), and the interactions of A*B, A*C, A*D, B*D, B*E and C*D were not found to be influential ($p > 0.05$). The concentration was found to be the most effective factor on the percentage inhibition of DPPH in comparison with Table 3. According to the single objective Taguchi Method, the 1.25% concentration of acorn leaf extract, 50% acetone, 50 °C and 45 min that was shown in the 8th experimental run of Table 2, was found to be suitable for DPPH inhibition.

DPPH is the most commonly applied antioxidant method as it is a simple and effective method for evaluating the antioxidant capacity of substances from plants and agro by-products [64]. In general, there is a definite correlation between the total phenolics and DPPH [65], and aqueous solvents have been reported to have higher DPPH activity than those of pure solvents [66]. Trabelsi et al. [58] examined the effect of certain solvents on antioxidant compounds of *L. monopetalum* leaves, and mixing pure solvents with water resulted in higher radical-scavenging activity than pure solvents. Also, they found that 80% acetone had the highest antiradical activity among solvents used in the study. In contrast, a study by Tabaraki et al. [67] was conducted to extract tannin from different acorn tissues, and they optimized the conditions of ultrasonic-assisted extraction by using a response surface methodology, which was 60 °C, 45 min and a methanol concentration of 74–82%. Both researchers reported a positive correlation between the total phenolics and DPPH. On the other hand, in a study on antioxidants and the cardioprotective potential of leaves of different acorn species, including *Quercus sideroxyla, Quercus eduardii* and *Quercus resinosa*, it was found that the highest total amount of phenolic material and DPPH activity was in the extract obtained with the acetone–water mixture (at 3:1) [56], however, they didn't report a positive correlation between the total phenolic content and DPPH. A similar result has also been found for the hazelnut kernels of different cultivars [68]. The absence of this relationship in the results obtained in the study could be related to the different

Table 6 Normalized S/N ratios for TPC, RSA, AA and principal component scores and their integrated MRPI

Experimental run	Normalized S/N ratios			Principal component scores			MRPI
	TPC	RSA	AA	PC1	PC2	PC3	
1	0.611172	0.990347	0.540768	1.066263	0.713755	−0.01959	0.944142
2	0.767359	0.952575	0.494302	1.130275	0.660275	−0.16459	0.969667
3	1	0.8397	0.918094	1.541374	0.413338	−0.0362	1.178088
4	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–
7	0.946728	0.803951	0.931591	1.503899	0.385594	0.009882	1.144761
8	0.67504	1	0.646359	1.183627	0.68731	0.009529	1.016829
9	0.804399	0.930846	0.788658	1.347484	0.565306	0.01511	1.092125
10	0.833082	0.872401	0.820637	1.371642	0.496914	0.015127	1.088003
11	0.972174	0.719416	1	1.542855	0.284463	0.036873	1.141269
12	0.622992	0.865033	0.538316	1.036428	0.592315	−0.03414	0.886884
13	0.653282	0.869841	0.721479	1.18181	0.549458	0.073177	0.974908
14	0.802957	0.813461	0.827287	1.338595	0.444404	0.039133	1.04991
15	0.965371	0.709512	0.891101	1.462004	0.301195	−0.03515	1.089994
16	0.829092	0.832606	0.843858	1.373068	0.454232	0.032873	1.076355
17	0.97159	0.696261	0.948672	1.501187	0.274194	0.000412	1.109152
18	0.627017	0.835643	0.574254	1.05488	0.555232	−0.01279	0.888619
19	0.515399	0.017189	0.483413	0.681182	−0.18689	−0.02613	0.408963
20	0.01084	0.743137	0	0.222186	0.708987	0.018501	0.366137
21	0.355602	0.443688	0.517023	0.71842	0.242064	0.126104	0.56608
22	0.47974	0.151667	0.518274	0.719285	−0.05985	0.028449	0.474145
23	0.079134	0.701947	0	0.25673	0.657335	−0.03149	0.373444
24	0.366886	0.481251	0	0.388653	0.394606	−0.24381	0.38128
25	0	0.752838	0	0.217617	0.720211	0.026548	0.366518
26	0.417615	0.444792	0.329086	0.634311	0.275122	−0.04996	0.515996
27	0.519774	0.06607	0.59032	0.770306	−0.16545	0.047588	0.477441
28	0.321718	0.524172	0.34343	0.601695	0.364966	0.031075	0.521983
29	0.555911	0	0.415253	0.657851	−0.19496	−0.10341	0.389455
30	0.125361	0.702949	0	0.288462	0.650005	−0.06432	0.392452
31	0.528715	0.037163	0.454715	0.676679	−0.16359	−0.05505	0.412498
32	0.093507	0.722572	0	0.272468	0.674489	−0.04098	0.389244
33	0.30483	0.542476	0.21783	0.510887	0.414325	−0.04451	0.473695
34	0.327556	0.507049	0.25293	0.539749	0.368304	−0.03726	0.479646
35	0.495805	0.160473	0.523878	0.736532	−0.05559	0.021278	0.487119
36	0.156351	0.679114	0	0.30265	0.621646	−0.08718	0.393263

Table 7 The results of eigen analysis of the correlation matrix

Principal component (PC)	Eigenvalue	Proportion explained (%)	Cumulative total (%)
1	2.0512	68.4	68.4
2	0.9053	30.2	98.6
3	0.0435	1.4	100

Table 8 The eigenvectors of the correlation matrix

Variable	Principal component		
	PC1	PC2	PC3
TPC	0.680	−0.179	−0.711
RSA	0.289	0.957	0.035
AA	0.674	−0.229	0.703

Table 9 Response table for means of MRPI values

Factors	Level 1	Level 2	Level 3	Max–Min	Rank
(A) Extract type	1.0434	0.4372	–	0.6062	1
(B) Temperature	0.7371	0.7390	0.7448	0.0077	5
(C) Time	0.7364	0.7382	0.7462	0.0098	4
(D) Solvent	0.7349	0.7274	0.7585	0.0311	3
(E) Concentration	0.6621	0.7729	0.7859	0.1238	2

polarity solvents and the extract was taken under different conditions (Table 2) so that the different phytochemicals could pass into the solvents. Thus, there may not be a difference in DPPH between extracts at different points.

Antimicrobial activity

The ANOVA results for the S/N ratios of the inhibition zones shown in Table 5 indicated that four factors and five interactions were found to be influential on the antimicrobial effects of the herbal extracts against *B. cereus* ($p < 0.05$). No significant differences were observed for solvent (D) and extract type–temperature (A*B) interaction ($p > 0.05$). Pursuant to the delta and rank values in Table 3, the extract type is the most significant factor, followed by concentration, temperature, solvent and time, respectively. As a result, the optimum conditions for antimicrobial activity are specified as follows: 5% concentration of acorn leaf extract, 50% methanol, 50 °C and 45 min, which corresponds to the 11th experimental run in Table 2. According to the results of the analysis, although the maximum value for the antimicrobial activity on *B. cereus* was obtained on the 11th run, when the variables including TPC, RSA and AA were evaluated simultaneously by PCA, it was determined that the third run was the optimum value.

Compared to green walnut husk, oak leaves have higher antimicrobial activity against *B. cereus*. The most critical factor in this antimicrobial activity may be attributed to the tannin compounds. Oak leaves are tannin-rich plant tissues [69]. The tannins have two forms: condensed and hydrolyzable tannins. With these compounds being secondary compounds, tannins can taste bitter [70]. Tannins have a protective effect against plant herbivores, and can grow on oak leaves as well as tasting bitter [24]. They also exhibit radical scavenging, antioxidant and antimicrobial activity [71]. Tian et al. [72] investigated the polarity effect of certain solvents on the antimicrobial and antioxidant activities of the extract obtained from *Galla chinensis*. As a result of this research, they noted that tannins are abundant in *Galina chinensis*, and the majority of the tannins from *Galina chinensis* were found to be non-polar or weak polar, and they also found that ethyl acetate, ethanol and 80% acetone extracts of it were effective against *Staphylococcus aureus, Bacillus subtilis*, and *Bacillus cereus* at 0.25 mg mL^{-1}, 0.5 mg mL^{-1} and 0.5 mg mL^{-1}, respectively. Moreno-Jimenez et al. [34] examined the antioxidant, anti-inflammatory and anticarcinogenic activity of red oak (*Quercus* spp.) leaves. They determined that the extract from *Quercus sideroxyla* leaves have a higher content of condensed tannin than the other two leaves, *Quercus durifolia* and *Quercus eduardii*. Moreover, the results of the study indicated that the extract from *Quercus sideroxyla* leaves had anti-inflammatory and antiproliferative effects. Gan et al. [73] investigated the identification and bioactivity of the basic gallotannins extracted from red beans, one of the hydrolyzable tannins. The extract was divided into nine different fractions which consisted of the I–V fractions eluted by 0–100% ethanol solutions and the others eluted by 40–100% acetone solutions. From these fractions, fraction VIII with acetone had the highest antibacterial activity against all tested bacteria, including *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (QAP D15), *Shigella flexneri* (QC 5820) and *Salmonella enterica serovar Typhimurium* (ATCC 14028), with inhibition zones of 11.9, 19.8, 12.9 and 12.6 mm, respectively.

Principal component analysis

The results of the single objective TM for each response obtained from the trials revealed that the method above is inadequate for detection of the joint optimum level for all responses. For this purpose, the PCA-based hybrid Taguchi approach was preferred for multi-response optimization/

Table 10 General linear ANOVA results for MRPI values

Factors	DF	Seq SS	Contribution (%)	Adj SS	Adj MS	F-value	p-Value
Extract	1	3.00658	94.21	3.00658	3.00658	859.24	0.000*
Concentration	2	0.09872	3.09	0.09872	0.04936	14.11	0.000*
Solvent	2	0.00492	0.15	0.00563	0.00281	0.80	0.460
Time	2	0.00040	0.01	0.00057	0.00029	0.08	0.922
Temperature	2	0.00037	0.01	0.00035	0.00017	0.05	0.952
Error	23	0.08048	2.52	0.08048	0.00350		
Total	32	3.19147	100.00				

*Significant

estimation. The normalized S/N ratios for each response were computed with Eq. (1) and displayed in Table 6. The calculated eigenvalues and eigenvectors of the correlation matrix are given in Tables 7 and 8, respectively. The principal component (PC) scores (PC1, PC2, and PC3) and their combined multi-response performance index (MRPI) values for each experimental run were acquired with Eqs. (2, 3) and shown in Table 6. The prediction of optimum levels for all factors was possible with a maximum MRPI value that corresponded to the factor levels of the third experimental run (5% concentration of acorn leaf extract, 50% acetone, 60 °C and 60 min) in Table 2. The factors affecting the levels of each parameter were ranked by means of all the MRPI values for every level that was indicated in Table 9. The results of the general linear ANOVA in Table 10 demonstrated the contribution of each factor in descending order as extract type, concentration, solvent, time and temperature, and the extract type and concentration were found to be the most significant ($p < 0.05$) of all the factors.

Conclusions

In this study, the use of the PCA-TM hybrid method has been attempted to find the solution to a multi-response estimation/optimization problem, in conjunction with a case investigation in both the fields of food and agricultural sciences. Furthermore, a comparison has been performed between single objective TM and PCA-TM hybrid methods for this problem. According to this study, the following conclusions can be declared:

(1) In single objective estimation with TM, different factors levels were found for each response. The predicted optimum factor settings for TPC, RSA and AA are 13333, 12231 and 12213, respectively. Also, the results of trials were confirmed by comparison with studies in published literature based on phytochemicals and herbal extracts.

(2) The results of the RSA analysis with a single objective TM showed that the inhibition percentage is inadequate to express the RSA of the studied herbal extracts with the mixed level orthogonal array design. It could be more suitable to use more reliable methods (e.g., Trolox equivalent antioxidant capacity assay [TEAC] or ascorbic acid equivalent, EC_{50}) instead of the percentage of inhibition.

(3) From the comparison of significant factors identified by ANOVA, only the extract type and concentration were found to be significant among the MRPI factors, while various factors and their interactions that are efficient, as identified by the single-objective TM method, are mentioned in the preceding sections. This kind of loss of expression or quality of some characteristics in a PCA-TM hybrid method is always probable when compared to a single objective TM [45]. However, the overall reliability was improved.

(4) In some cases, the use of PCA can be disadvantageous if there is more than one principal component whose eigenvalue is > 1, and the solution of this shortcoming is unknown [43]. However, there was only one eigenvalue found, which was used for the calculation of MRPI values, to be higher than 1 (Table 7).

(5) In a PCA-based Taguchi hybrid method, an optimal factor setting was determined with the MRPI for all responses corresponding to the factor levels (13333) of the third experimental run in the design matrix. This method has proved itself from the point of the estimation of optimal levels in this study. However, it is necessary to probe the usage of the PCA-TM hybrid method in the field of both food and agricultural sciences by considering different conditions and factors.

Acknowledgements We would like to thank ERU-TAUM (Erciyes University, Technology Research and Application Center) for letting us use their laboratory-type freeze-dryer.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. K.W. Wahle, I. Brown, D. Rotondo, S.D. Heys, Adv. Exp. Med. Biol. **698**, 36 (2010)
2. S. Jafari, S. Saeidnia, M. Abdollahi, Curr. Pharm. Biotechnol. **15**(4), 409 (2014)
3. M. Carocho, M.F. Barreiro, P. Morales, I.C.F.R. Ferreira, Compr. Rev. Food Sci. Food Saf. **13**(4), 377 (2014)
4. WHO, Antibiotic resistance. http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/. Accessed 5 Feb 2018
5. A. Moure, J.M. Cruz, D. Franco, J.M. Dominguez, J. Sineiro, H. Dominguez, M.J. Nunez, J.C. Parajo, Food Chem. **72**(2), 145 (2001)
6. N. Balasundram, K. Sundram, S. Samman, Food Chem. **99**(1), 191 (2006)
7. F. Kallel, D. Driss, F. Chaari, L. Belghith, F. Bouaziz, R. Ghorbel, S.E. Chaabouni, Ind. Crops Prod. (2014) https://doi.org/10.1016/j.indcrop.2014.07.047
8. O.H. Lee, B.Y. Lee, Bioresour. Technol. **101**(10), 3751 (2010)
9. B. Melgar, M.I. Dias, A. Ciric, M. Sokovic, E.M. Garcia-Castello, A.D. Rodriguez-Lopez, L. Barros, I. Ferreira, Ind. Crops Prod. **107**, 353 (2017)
10. J.L. McKillip, Antonie Van Leeuwenhoek **77**(4), 393 (2000)
11. A. Osimani, L. Aquilanti, F. Clementi, Int. J. Hosp. Manag. **72**, 145 (2018)
12. A. Tewari, S. Abdullah, J. Food Sci. Technol. **52**(5), 2500 (2015)
13. E.J. Bottone, Clin. Microbiol. Rev. **23**(2), 382 (2010)
14. P.E. Granum, in *Applications and systematics of Bacillus and relatives*, ed. by R. Berkeley, M.N. Heyndrickx, P. de Vos (Blackwell, Oxford, 2002), p. 37

15. H. Jun, J. Kim, J. Bang, H. Kim, L.R. Beuchat, J.H. Ryu, Int. J. Food Microbiol. **160**(3), 260 (2013)
16. L.R. Beuchat, in *Microbial Food Contamination*, ed. by C.L. By, S. Wilson, Droby (CRC Press, Boca Raton, 2000), p. 149
17. T.K. Lim, *Edible Medicinal and Non-medicinal Plants*, vol. 3 (Springer, New York, 2012), pp. 70–72
18. J. Azmir, I.S.M. Zaidul, M.M. Rahman, K.M. Sharif, A. Mohamed, F. Sahena, M.H.A. Jahurul, K. Ghafoor, N.A.N. Norulaini, A.K.M. Omar, J. Food Eng. **117**(4), 426 (2013)
19. B.K. Tiwari, Trac-Trend Anal. Chem. **71**, 100 (2015)
20. A. Phaniendra, D.B. Jestadi, L. Periyasamy, Indian J. Clin. Biochem. **30**(1), 11 (2015)
21. V. Lobo, A. Patil, A. Phatak, N. Chandra, Pharmacogn. Rev. **4**(8), 118 (2010)
22. H.M. Ali, A. Abo-Shady, H.A.S. Eldeen, H.A. Soror, W.G. Shousha, O.A. Abdel-Barry, A.M. Saleh, Chem. Cent. J. **7**(1), 53 (2013)
23. M.J. Alves, I.C. Ferreira, H.J. Froufe, R. Abreu, A. Martins, M. Pintado, J. Appl. Microbiol. **115**(2), 346 (2013)
24. J.P. Salminen, T. Roslin, M. Karonen, J. Sinkkonen, K. Pihlaja, P. Pulkkinen, J. Chem. Ecol. **30**(9), 1693 (2004)
25. C. Moctezuma, A. Hammerbacher, M. Heil, J. Gershenzon, R. Méndez-Alonzo, K. Oyama, J. Chem. Ecol. **40**(5), 458 (2014)
26. M. Gulluce, A. Adiguzel, H. Ogutcu, M. Sengul, I. Karaman, F. Sahin, Phytother. Res. **18**(3), 208 (2004)
27. V. Nour, I. Trandafir, S. Cosmulescu, J. Chromatogr. Sci. **51**(9), 883 (2013)
28. M. Carvalho, P.J. Ferreira, V.S. Mendes, R. Silva, J.A. Pereira, C. Jeronimo, B.M. Silva, Food Chem. Toxicol. **48**(1), 441 (2010)
29. A. Fernandez-Agullo, E. Pereira, M.S. Freire, P. Valentao, P.B. Andrade, J. Gonzalez-Alvarez, J.A. Pereira, Ind. Crops Prod. **42**, 126 (2013)
30. F. Stampar, A. Solar, M. Hudina, R. Veberic, M. Colaric, Food Chem. **95**(4), 627 (2006)
31. B.B. Shi, W.N. Zhang, X. Li, X.J. Pan, Int. J. Food Prop. **20**, S2635 (2018)
32. I. Oliveira, A. Sousa, I.C. Ferreira, A. Bento, L. Estevinho, J.A. Pereira, Food Chem. Toxicol. **46**(7), 2326 (2008)
33. N.E. Rocha-Guzman, J.A. Gallegos-Infante, R.F. Gonzalez-Laredo, R. Reynoso-Camacho, M. Ramos-Gomez, T. Garcia-Gasca, M.E. Rodriguez-Munoz, S.H. Guzman-Maldonado, L. Medina-Torres, B.A. Lujan-Garcia, Food Chem. **115**(4), 1320 (2009)
34. M.R. Moreno-Jimenez, F. Trujillo-Esquivel, M.A. Gallegos-Corona, R. Reynoso-Camacho, R.F. Gonzalez-Laredo, J.A. Gallegos-Infante, N.E. Rocha-Guzman, M. Ramos-Gomez, Food Chem. Toxicol. **80**, 144 (2015)
35. M.H. Xu, X.Y. Yang, M.R. Fu, Waste Biomass Valoriz. **7**(5), 1159 (2016)
36. P.S. Makkar, B. Singh, Bioresour. Technol. **37**(2), 185 (1991)
37. M.T. Moradi, A. Karimi, S. Alidadi, Chin. J. Nat. Med. **14**(3), 196 (2016)
38. A. Bevilacqua, M. Corbo, M. Sinigaglia, *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (Formatex Research Center, Badajoz, 2010), p. 1419
39. R.K. Roy, *A Primer on the Taguchi Method*, 2nd edn. (Society of Manufacturing Engineers, USA, 2010), pp. 9–24
40. R.S. Rao, C.G. Kumar, R.S. Prakasham, P.J. Hobbs, Biotechnol. J. **3**(4), 510 (2008)
41. M.H. Oztop, S. Sahin, G. Sumnu, J. Food Eng. **79**(1), 83 (2007)
42. Minitab Getting Started with Minitab 18. https://www.minitab.com/uploadedFiles/Documents/getting-started/MinitabGettingStarted_EN.pdf. Accessed 5 Feb 2017
43. R. Jeyapaul, P. Shahabudeen, K. Krishnaiah, Int. J. Adv. Manuf. Technol. **26**(11–12), 1331 (2005)
44. J. Antony, Qual. Reliab. Eng. Int. **16**(1), 3 (2000)
45. A.K. Dubey, V. Yadava, Opt. Lasers Eng. **46**(2), 124 (2008)
46. T. Yih-Fong, C. Fu-Chen, Int. J. Mach. Mach. Mater. **1**(1), 76 (2006)
47. R.L. Bradley, in *Food Analysis*, ed. by S.S. By, Nielsen (Springer, Boston, 2010), p. 85
48. H. Abdi, L.J. Williams, Wiley Interdiscip. Rev. Comput. Stat. **2**(4), 433 (2010)
49. M. Hayta, E.M. Iscimen, LWT-Food Sci. Technol. **77**, 208 (2017)
50. K. Gezer, M. Duru, I. Kivrak, A. Turkoglu, N. Mercan, H. Turkoglu, S. Gulcan, Afr. J. Biotechnol. **5** (20), 1924 (2006)
51. H. Baydar, O. Sağdiç, G. Özkan, T. Karadoğan, Food Control **15**(3), 169 (2004)
52. A. Soto-Vaca, A. Gutierrez, J.N. Losso, Z.M. Xu, J.W. Finley, J. Agric. Food Chem. **60**(27), 665 (2012)
53. K.M. Hammi, A. Jdey, C. Abdelly, H. Majdoub, R. Ksouri, Food Chem. **184**, 80 (2015)
54. J.A. Sanchez-Burgos, M.V. Ramirez-Mares, M.M. Larrosa, J.A. Gallegos-Infante, R.F. Gonzalez-Laredo, L. Medina-Torres, N.E. Rocha-Guzman, Ind. Crops Prod. **42**, 57 (2013)
55. B.M. Popovic, D. Stajner, R. Zdero, S. Orlovic, Z. Galic, Sci. World J. (2013) https://doi.org/10.1155/2013/134656
56. M.J. Rivas-Arreola, N.E. Rocha-Guzman, J.A. Gallegos-Infante, R.F. Gonzalez-Laredo, M. Rosales-Castro, J.R. Bacon, R. Cao, A. Proulx, P. Intriago-Ortega, Pak. J. Biol. Sci. **13**(11), 537 (2010)
57. M. Markom, M. Hasan, W.R.W. Daud, H. Singh, J.M. Jahim, Sep. Purif. Technol. **52**(3), 487 (2007)
58. N. Trabelsi, W. Megdiche, R. Ksouri, H. Falleh, S. Oueslati, B. Soumaya, H. Hajlaoui, C. Abdelly, LWT-Food Sci. Technol. **43**(4), 632 (2010)
59. M. Ahmed, H. Fatima, M. Qasim, B. Gul, BMC Complement Altern. Med. **17**, 386 (2017)
60. C. Alasalvar, M. Karamac, R. Amarowicz, F. Shahidi, J. Agric. Food Chem. **54**(13), 4826 (2006)
61. A. Mokhtarpour, A.A. Naserian, R. Valizadeh, M. Danesh Mesgaran, Annu. Rev. Res. Biol. **4**(8), 1330 (2014)
62. R. Tabaraki, S. Rastgoo, Korean J. Chem. Eng. **31**(4), 676 (2014)
63. K. Ghasemi, Y. Ghasemi, A. Ehteshamnia, S.M. Nabavi, S.F. Nabavi, M.A. Ebrahimzadeh, F. Pourmorad, J. Med. Plant Res. **5**(7), 1128 (2011)
64. S.B. Kedare, R. Singh, J. Food Sci. Technol. **48**(4), 412 (2011)
65. N. Pawar, S. Pai, M. Nimbalkar, G. Dixit, Food Chem. **126**(3), 1330 (2011)
66. I.F. Almeida, M.H. Amaral, P.C. Costa, M.F. Bahia, P. Valentão, P.B. Andrade, R.M. Seabra, T.M. Pereira, Biofactors **33**(4), 267 (2008)
67. R. Tabaraki, A. Safari, F.A. Yeganeh, J. Appl. Chem. Res. **7**(3), 67 (2013)
68. J. Jakopic, M. Mikulic-Petkovsek, A. Likozar, A. Solar, F. Stampar, R. Veberic, Food Chem. **124**(3), 1100 (2011)
69. M.Y. Elahi, Y. Rouzbehan, Anim. Feed Sci. Technol. **140**(1–2), 78 (2008)
70. R. Amarowicz, A. Troszynska, N. Barylko-Pikielna, F. Shahidi, J. Food Lipids **11**(4), 278 (2004)
71. K.T. Chung, T.Y. Wong, C.I. Wei, Y.W. Huang, Y. Lin, Crit. Rev. Food Sci. Nutr. **38**(6), 421 (1998)
72. F. Tian, B. Li, B. Ji, G. Zhang, Y. Luo, LWT-Food Sci. Technol. **42**(7), 1289 (2009)
73. R.Y. Gan, K.W. Kong, H.B. Li, K. Wu, Y.Y. Ge, C.L. Chan, X.M. Shi, H. Corke, Front. Chem. **6**, 39 (2018)