



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Optimisation of phenolic compounds and antioxidant activity extraction conditions of a roasted mix of *Tetrapleura tetraptera* (Schumach & Thonn.) and *Aframomum citratum* (C. Pereira) fruits using response surface methodology (RSM)

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ARTICLE INFO

Article history:

Received 20 October 2019

Revised 30 April 2020

Accepted 3 May 2020

Available online 8 May 2020

Keywords:

Optimization

Roasting

Polyphenols

*Tetrapleura tetraptera**Aframomum citratum*

ABSTRACT

The therapeutic abilities of *Tetrapleura tetraptera* and *Aframomum citratum* fruits used as spices are attributed to their bioactive molecules, including polyphenols. Sometimes used together and heated, they can undergo denaturation. The aim of the current study is to optimize the extraction of phenolic compounds and antioxidant potential of a roasted mix of *Tetrapleura tetraptera* and *Aframomum citratum* (95/5: w/w) fruits using RSM in a home food consumption context. The mix of spices was chosen according to the highest content of TPP and preliminary studies were performed to select the influencing variables. Roasting temperatures (130–170 °C), roasting times (10–15 min) and brewing times (8–15 min) were investigated with a rotatable central composite design. Experimental results were fitted to the second-order polynomial model where multiple regressions and ANOVA were used to determine the coefficients of the model and the optimal conditions for the considered responses. The two spices are good sources of phenolic compounds, and they also show significant ($p < 0.05$) dose-dependent radical scavenging activities (DPPH assay and inhibition of β -carotene discoloration) and reductive activities (FRAP assay and Phosphomolybdenum method). They significantly inhibit bovine serum albumin and 5-LOX denaturation. Brewing time and roasting time significantly ($p < 0.05$) influence the responses and there is a strong ($R^2 = 0.93$) correlation between the TPP and TAC of the beverage. The quadratic model fit well and the different factors used to test its accuracy and fitness were in satisfactory ranges. For TPP extraction (38.90 mgGAE/g dw) and TAC (50.75 mg TE/g dw) expression, the optimal conditions were reached at a roasting temperature of 150 °C, roasting time of 12.62 min, brewing time of 11.91 min and a desirability of 0.95. The novel information on the optimisation of the process can be further used by scientists, consumers and herbalists for effective handling of fruits during the extraction process.

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1. Introduction

Tetrapleura tetraptera and *Aframomum citratum* fruits have been identified among the spices that are most commonly used and

available in Cameroonian cities. *T. tetraptera* is from the rainforest of West African (Eyog-Matig et al., 2006) and it belongs to the class of Magnoliopsida, Fabaceae family. *Aframomum citratum* is part of Angiosperms, family of zingiberaceae, endemic of tropical flora of sub-Saharan Africa and Madagascar (Abondo et al., 1995). Mostly harvested in the forest, they show their importance locally by their contribution to many feeding habits, in few sub-Saharan countries (Nigeria, Ghana, Ivory-Coast, Zambia, Congo, and Benin) where they are commonly used together for many traditional recipes (Akin-Idowu et al., 2011; Djiazet et al., 2016). They also contribute to the local pharmacopeia. Indeed, fruits, leaves, roots, stems and bark of the different plant species are used to treat many pathological states including hypertension, malaria, epilepsy, schistosomia-

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Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sjbs.2020.05.003>

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sis, cough, post-partum contractions, and fertility (Moukette et al., 2015). They also have anti-arthritis, anticancer, anti-inflammatory and anti-diabetic properties (Ojewole and Adewunmi, 2004; Ozaslan et al., 2016). The therapeutic attributes of those fruits are due to the presence of bio-active compounds (alkaloids, flavonoids, saponins, tannins, phenols and glycosides) which are essential for health (Etoundi et al., 2010; Akin-Idowu et al., 2011). As aromatic plants, the fruits contains also essential oils, primarily carboxylic acids and terpenes (Meffo et al., 2019; Udourioh and Tokudoh, 2014). Most of these compounds possess potential antioxidant activity in the same way as vitamins (Vitamin C and E) and mineral content (Fe, Zn) of the two plant species (Akin-Idowu et al., 2011; Ironi et al., 2016).

The hard-shelled fruits of *T. tetraptera* and *A. citratum* are mostly roasted, burned or heated before being used during cooking to facilitate the grinding and release of aromas. For medical purposes, solvent extraction is the most common technique employed to obtain extracts (Jiofack et al., 2010). However, the efficiency of the extraction procedure is determined by the plant matrix, bioactive components collected and the process used. More specifically, Gunathilake et al. (2018) reported that the extraction of a compound is influenced by factors including temperature, time, solvent polarity, the size of particles, extraction methods, etc. Indeed, Horváthová et al. (2007) stated that heat treatment causes changes in the integrity of the plant cell structure, which would lead to positive and/or negative effects on the antioxidant activity of its constituents. It has also been reported that all spices are hydro-distilled in most cooking modes, but that overheating results in a significant loss of chemical elements depending on cooking times, households, and the user's culture (Nwaichi and Anyanwu, 2013). These negative effects would increase with longer cooking times (Abu-Ghannam and Jaiswal, 2015) and would be all the more detrimental as the average cooking times of meals are beyond 40 min.

Infusion, maceration or decoction are traditionally used when preparing therapeutic recipes (Jiofack et al., 2010). Among these extraction methods, infusion is the fastest and most effective way to obtain plant extracts and it has the advantage of presenting the most important extraction yields that are positively correlated with the content of bioactive compounds for very short extraction times (Adouni et al., 2018). Preparing a rapid beverage with a mixture of these spices could reduce the adverse effects of heat on the bioactive compounds. In the context of domestic food consumption, an optimal extraction method has not been developed for the two plant species, including for a mixture. The lack of reports of heat effects on the bioactive compounds of these fruits is a real handicap to the mastery of the technological and therapeutic processes that use them. Therefore, the global aim of the current study was to define the optimal conditions for phenolic compounds extraction and antioxidant activity of a mixture of the two roasted spices. To achieve this goal, the response surface methodology (RSM) design was used to establish the model and optimise the extraction conditions. However preliminary tests have been achieved to determine few phytochemical characteristics and biological activities of the spices; and to determine the extraction parameters.

2. Material and methods

2.1. Collecting, authentication and handling of fruits

T. tetraptera and *A. citratum* fresh fruits were harvested in Bonda (12°0'42.6960"E) and Ngato (15°2'50.8215"E) localities in Cameroon, respectively. Each specimen was identified by Dr Fongnzossie Evariste, botanist at Douala University, and voucher specimens (N°31610/NHC, N°37795/NHC respectively) were

deposited in the National Herbarium for further reference. Previously cleaned and sorted, they were dried at 45 °C for 72 h (Memmert, UF160), before being vacuum packed and kept at 4 °C for further analysis.

2.2. Roasting of fruits

Dried fruits were sliced in equal small sizes (1 cm²), before being mixed and roasted using a home coffee roaster Machine (Vingloo, 1200w; 110/220 V), according to the experimental design. Cooled at room temperature, roasted spices were ground (IKA, M20) and the powder was passed through a sieve (0.425 mm). The powder retained was vacuum packed (Henkelman, Boxer 35) and stored at 4 °C for further analysis.

2.3. Total polyphenols (TPP) and total flavonoids content (TFL) analysis

All solvents and reagents at analytical grade were purchased from Sigma-Aldrich Co (Belgium) and Sigma Chemical (St Louis, MO, USA). For both fruits and the formulation, TPP content was assessed using the Folin-Ciocalteu colorimetric method as described by Moukette et al. (2015) with slight modifications and the amounts were calculated from the Gallic Acid standard curve (0.05–1 mg/mL), and expressed as mg GAE/g dw (Gallic Acid Equivalent/ g dry weight). TFL content was also quantified (Alara et al., 2017) from the Catechin standard curve (0.05–1 mg/mL) and expressed as mg/g dry weight of Catechin Equivalent (mg CE/g dw).

2.4. Antioxidant activities measurements

2.4.1. Free radical scavenging activities

The methods used for this study were based on DPPH (2,2-Diphenyl-1-picrylhydrazyl radical) trapping assay (Alara et al., 2017) and on the inhibition of β-carotene discoloration (Bougandoura and Bendimerad, 2013). DPPH discoloration by extracts (at concentrations 0.25, 2.5, 25, 250 mg/mL) was monitored at 517 nm after 30 min of incubation in the dark at room temperature. The IC₅₀ (half maximal inhibitory concentration) of each sample was obtained graphically and the method was standardised by Ascorbic acid. The inhibition of β-carotene discoloration by the extracts (250 mg/mL) was followed at 490 nm after 24 h against BHT (Butylated hydroxytoluene) prepared under the same conditions.

2.4.2. Reducing activities of fruits

The reducing power of the different extracts (0.25, 2.5, 25, 250 mg/mL) was measured by the "Ferric Reducing Antioxidant Power" or FRAP (Vijayalakshmi and Ruckmani, 2016) and by the Phosphomolybdenum method or TAC (Prieto et al., 1999). For the first test, ascorbic acid was used as a positive control and the reducing activity was expressed as mg AAE/g dw (mg Ascorbic acid equivalent) of extract. The TAC is expressed as mg TE/g dw (milligram Trolox Equivalent) and was based on a Trolox standard curve.

2.5. Anti-inflammatory activities of fruits

Protein (Bovine Serum Albumin) denaturation and lipoxygenase (5-LOX) inhibition by the extracts (0.25, 2.5, 25, 250 mg/mL) was performed (Gunathilake et al., 2018). Absorbance measurements for the first test were made at 660 nm and diclofenac was used as a positive control. For the second test, the absorbance was measured at 234 nm, phosphate buffer solution was used as a negative control and Quercetin was a positive control. The inhibition per-

centages were calculated and the extract IC₅₀ values were obtained by linear regression.

2.6. Optimisation of extraction process

2.6.1. Formulation test

For that preliminary assay, four formulations (95/5, 90/10, 85/5, 80/20: w/w between *T. tetraptera* and *A. citratum*) were prepared before heat treatment and examined in order to choose the one with the highest content of total polyphenols. To prepare the plant extracts, four extractions ratio (1/1; 1/2; 1/3, 1/4: weight/volume) were tested and water was chosen as the solvent.

2.6.2. Screening of optimization factors

Roasting as a pre-treatment is a prerequisite in general to the use of these spices in the context of domestic food consumption. Initially, the influencing parameters were selected by preliminary experiments on the basis of single factor multiple levels. Roasting temperatures (100, 120, 140, 160, 180, 200, 220 and 240 °C) at a fixed roasting time (5 min), roasting times (5, 10, 15, 20, 25 and 30 min) at the maximum roasting temperature obtained, and brewing times (4, 8, 12, 16 and 20 min) at the maximum roasting temperature and time obtained were studied. For each successive test, TPP was analysed on fruit extracts.

2.6.3. Experimental design

After the preliminary tests, the experimental ranges of the independent variables were defined and are presented in Table 1. The variables are coded (xi) according to the following equation:

$$xi = \frac{(Xi - Xo)}{\Delta X} \quad (1)$$

Xi: actual value of the independent variable; Xo: real value of an independent variable at the centre point and ΔX is the step change value.

A rotatable central composite design (RCCP) consisting of 20 experimental runs including 8 tests of the factorial design, 6 star tests and 6 repetitions in the centre was assessed. The minimum and maximum levels of the star points were −1.68 and +1.68, respectively. Thus, each factor (X₁, X₂, X₃) was studied for the five levels (−1.68; −1; 0; 1; +1.68). All experiments were conducted in triplicate and the data were analysed using the MINITAB 18 software (Pennsylvania, PA, USA).

2.7. Statistical analysis and model validation

Optimisation of the extraction of antioxidant components was carried out by using response surface methodology (RSM). RSM is a combination of statistical and mathematical techniques used for optimizing many processes. In this case, optimum process parameters were achieved by maximizing total polyphenols (TPP) content and simultaneously total antioxidant capacity (TAC) of the plant extract. During the optimization stage, the desirability

function of the MINITAB statistical software was used to obtain the best compromise of the two responses with the weights of all 1.0. ANOVA and Duncan's multiple test were used to determine significant differences in TPP content and the TAC of beverages ($p < 0.05$). Contour plots were designed using the same software.

To determine the average TPP and TAC values of the beverage, a regression model containing ten coefficients including linear and quadratic effects on factors and linear effects on interactions was described using the following equation:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{j=1}^k \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i < j}}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j \quad (2)$$

K: number of variables; β_0 : intercept; β_i : linear coefficient for the main factor; β_{ii} : the quadratic coefficient for the main factors; β_{ij} : the second order interaction coefficient; *Y*: predicted TPP content and TAC of the beverage.

The adequacy of the regression model generated by the factorial design was examined using the following indicators: ANOVA ($p < 0.05$) of the postulated model, the determination coefficient (R^2), absolute average deviation (ADD), bias factor (B_f), accuracy factor A_f , and complementary experience (Schubert et al., 2010). AAD, B_f , A_f were mathematically determined using the following equations:

$$AAD = \frac{\sum_{i=1}^N \left(\frac{|Y_{i,obs} - Y_{i,cal}|}{Y_{i,obs}} \right)}{N} \quad (3)$$

$$B_f = 10^{\frac{1}{N} \sum_{i=1}^N \log \left(\frac{Y_{i,cal}}{Y_{i,obs}} \right)} \quad (4)$$

$$A_f = 10^{\frac{1}{N} \sum_{i=1}^N \left| \log \left(\frac{Y_{i,cal}}{Y_{i,obs}} \right) \right|} \quad (5)$$

$Y_{i,obs}$: The observed experimental response; $Y_{i,cal}$: The predicted response; *N*: Total number of experiments

3. Results and discussion

3.1. Total polyphenols and total flavonoids contents

TPP content was calculated from the linear regression curve $y = 0.7726x + 0.002$ ($R^2 = 0.99$), while TFL content was calculated from the following linear regression curve: $y = 0.0151x + 0.0098$ ($R^2 = 0.99$). The two spices are good sources of TPP and TFL (Table 2), and *T. tetraptera* fruits are more than five times richer than *A. citratum* fruits. The result obtained was confirmed by (Etoundi et al., 2010), who demonstrated that the TPP of *T. tetraptera* extracts was 5 times greater than that of *A. citratum* per milligram of plant powder. The amount of TPP registered for *T. tetraptera* was not different to that noted by Akin-Idowu et al.

Table 1
Independent variables (actual and coded values).

Independent variables	Symbols	Coded level		
		Low level (−1)	Center value (0)	High level (+1)
Roasting Temperature (°C)	X ₁	130	150	170
Roasting time (min)	X ₂	10	12.5	15
Brewing time (min)	X ₃	8	11.5	15

Table 2
Few phytochemical characteristics of *A. citratum* and *T. tetraptera* fruits.

Species	TPP (mg GAE/g dw)	TFL (mg CE/g dw)
<i>T. tetraptera</i>	20.64 ± 0.35 ^b	0.71 ± 0.15 ^a
<i>A. citratum</i>	4.82 ± 0.31 ^c	0.14 ± 0.07 ^b
<i>T. tetraptera/A. citratum</i> (95/5)	22.13 ± 0.14 ^a	0.77 ± 0.04 ^a

Means ± standard deviations of three observations followed by different superscripts (a,b in the same column are significantly different at 5% level; GAE: Gallic acid equivalent; CE: Catechin equivalent; dw: dry weight; TPP: Total Polyphenols; TFL: Total Flavonoids.

(2011), but was lower than the quantity reported by Sokamte et al. (2019). *A. citratum* TPP was lower than that noted by Nguélé et al. (2012). The findings for *T. tetraptera* characteristics (fat, protein, sugar and ash content) were not different to previous reports (Ironi et al., 2016; Ironi et al., 2013; Uhegbu et al., 2011).

3.2. In vitro biological activities of spices

All of the results on the biological activities of extracts are presented in Table 3. The extracts contain electron donors that significantly ($p < 0.05$) inhibit DPPH (1,1-Diphényl-2-Pyryl Hydrazil) radicals and the inhibition percentage is dose-dependent. *T. tetraptera* extracts present a scavenging power that is at least 3 times greater than that recorded by *A. citratum* extracts at the same concentration. The concentrations of extract needed to decrease the initial DPPH concentration by 50% are 4.85, 14 and 4.14 times greater than the reference IC₅₀ (0.07 g/mL) for *T. tetraptera*, *T. tetraptera/A. citratum* and *A. citratum*, respectively. The results are similar to those which have already been published (Etoundi et al., 2010; Sokamte et al., 2019). Additionally, the highest activities were noticed in spices (for both plants species) with the most important TPP and TFL contents, as reported by Dai and Mumper (2010). It is clear that polyphenols have an ideal chemistry struc-

ture for free radical elimination, and therefore have a significant antioxidant activity. The presence of the extracts also significantly ($p < 0.05$) inhibits β-carotene discoloration and *T. tetraptera/A. citratum* extracts are more effective after 24 h than the individual spice effects but less effective than the reference (BHT). β-carotene discoloration inhibition by the plant extracts gives them good potential in the food industry and in medicinal fields because they can control oxidation in food systems and protect them (food safety implications) due to the importance of carotenoids or provitamin A in the diets (Marc et al., 2004).

The different extracts also possess reductive activity. Indeed, the iron-reducing activity is dose-dependent and the *T. tetraptera/A. citratum* extract is the most effective for each concentration tested. Evaluating the antioxidant activity of the extracts using the Phosphomolybdenum method revealed that *A. citratum* extracts are 6.58 times less effective than the other extracts. At 250 mg/mL, the results have the same tendency as previous reports (Moukette et al., 2015) on the aqueous and organic extracts, even at lower concentrations with respect to the TAC activity. However, the iron-reducing power presented by (Sokamte et al., 2019) were contrary to the current ones. They recorded a higher reducing ability for the *A. citratum* methanolic extract than the *T. tetraptera* extract (154.43 ± 3.02 against 56.53 ± 0.66 mg AAE/g). The reducing ability of these extracts could be explained by the presence of reductones (Dai and Mumper, 2010) causing the formation of hydrogen peroxide. With regard to *in vitro* anti-inflammatory activities, the plant extracts have significant ($p < 0.05$) anti-inflammatory activity which is dose-dependent but lower than that of diclofenac and quercetin, respectively, for the inhibition of BSA denaturation and 5-LOX activity. *A. citratum* extracts are less effective than the other extracts and there is no significant difference between *T. tetraptera/A. citratum* and *T. tetraptera* activities. Therefore, their use in traditional medicine are justified for inflammatory diseases (Ojewole and Adewunmi, 2004; Etoundi et al., 2010; Ozaslan et al., 2016) and the results obtained are related

Table 3
Biological activities of *A. citratum* and *T. tetraptera* fruits.

Biological activities	Species and references	Extracts concentrations (mg/mL)				Extracts IC ₅₀ (g/mL)
		0.25	2.5	25	250	
<i>Antioxidant activities</i>						
DPPH inhibition (%)	<i>T. tetraptera</i>	3.08 ± 0.68	11.58 ± 0.75	38.29 ± 0.70	67.52 ± 0.50	0.34 ± 0.10 ^c
	<i>A. citratum</i>	0.01 ± 0.03	2.05 ± 0.32	8.38 ± 1.00	17.35 ± 0.01	0.98 ± 0.22 ^d
	<i>T. tetraptera/A. citratum</i> (95/5)	3.94 ± 0.46	12.14 ± 0.37	41.31 ± 0.22	69.04 ± 0.37	0.29 ± 0.17 ^b
	Ascorbic acid	11.40 ± 0.73	27.37 ± 0.06	61.34 ± 0.63	85.67 ± 0.90	0.07 ± 0.00 ^a
FRAP (mg AAE/g dw)	<i>T. tetraptera</i>	1.11 ± 0.38	5.09 ± 0.43	19.63 ± 0.17	24.50 ± 0.03	
	<i>A. citratum</i>	0.00 ± 0.03	0.67 ± 0.08	15.20 ± 0.55	22.35 ± 0.50	
	<i>T. tetraptera/A. citratum</i> (95/5)	1.41 ± 0.04	6.11 ± 0.27	22.54 ± 0.09	27.46 ± 0.13	
β-carotene discoloration inhibition (%)	<i>T. tetraptera</i>				73.61 ± 0.80 ^c	
	<i>A. citratum</i>				62.40 ± 0.87 ^d	
	<i>T. tetraptera/A. citratum</i> (95/5)				78.15 ± 0.38 ^b	
	BHT				94.25 ± 0.19 ^a	
TAC (mg TE/g dw)	<i>T. tetraptera</i>				29.81 ± 0.72 ^a	
	<i>A. citratum</i>				4.53 ± 0.13 ^b	
	<i>T. tetraptera/A. citratum</i> (95/5)				31.95 ± 0.2 ^a	
<i>Anti-inflammatory activities</i>						
BSA denaturation inhibition (%)	<i>T. tetraptera</i>	6.12 ± 0.03	11.67 ± 0.13	29.84 ± 0.24	82.55 ± 0.08	0.14 ± 0.03 ^{bc}
	<i>A. citratum</i>	4.68 ± 0.25	8.56 ± 0.23	12.50 ± 0.00	61.61 ± 0.19	0.22 ± 0.01 ^c
	<i>T. tetraptera/A. citratum</i> (95/5)	7.08 ± 0.07	13.04 ± 0.21	35.52 ± 0.32	85.85 ± 0.20	0.12 ± 0.01 ^b
	Diclofenac	12.74 ± 0.31	36.33 ± 0.21	82.82 ± 0.42	97.22 ± 0.41	0.05 ± 0.00 ^a
5-LOX denaturation inhibition (%)	<i>T. tetraptera</i>	19.22 ± 0.08	40.68 ± 0.40	67.25 ± 0.45	80.59 ± 0.48	0.11 ± 0.01 ^b
	<i>A. citratum</i>	13.36 ± 0.27	30.38 ± 0.37	46.83 ± 0.10	57.07 ± 0.27	0.20 ± 0.02 ^c
	<i>T. tetraptera/A. citratum</i> (95/5)	19.92 ± 0.11	44.27 ± 0.15	69.95 ± 0.27	82.73 ± 0.37	0.10 ± 0.04 ^b
	Quercetin	44.18 ± 0.17	65.43 ± 0.56	90.53 ± 0.44	98.97 ± 0.60	0.04 ± 0.02 ^a

Means ± standard deviations of three observations followed by different letters (a,b,c) in the same column are significantly different at 5% level.

AAE: Ascorbic acid equivalent; TE: Trolox equivalent; BHT: Butyl hydroxytoluène; TAC: Total antioxidant capacity; FRAP: Ferric Reducing Antioxidant Power; DPPH: 1,1-Diphenyl 2 Pyryl Hydrazil; BSA: Bovine Serum Albumin; IC₅₀: Half maximal inhibitory concentration; 5-LOX: lipoxygenase.

to the physicochemical content of extracts (Moukette et al., 2015; Sokamte et al., 2019), in particular phenolic acids, flavonoids, minerals, vitamins etc. Indeed, the authors have demonstrated the presence of phenolic acids (benzoic, vanillic, coumaric, syringic, caffeic, gallic, chlorogenic, ellagic) and flavonoids (apigenin, catechin, epicatechin, rutin, quercetin, luteolin) in *T. tetraptera* extracts (Moukette et al., 2015), while *A. citratum* extracts are reported to contain Ferulic acid, t-cinnamic acid, epicatechin, quercetin and eugenol (Sokamte et al., 2019). Flavonoids are known to act on the inflammatory response via many routes by blocking molecules like cyclooxygenase, iNOS (inducible nitric oxide synthase protein), cytokines, nuclear factor- κ B and matrix metalloproteinases and they are good free radical scavengers that donate hydrogen, inhibit LPO (lipid peroxidation) and act as metal ion chelators (Amadi et al., 2016). Meffo et al. (2019) also reported that the main component of the essential oils of *A. citratum* is geraniol. Geraniol was found to significantly decrease lipid peroxidation, inhibit nitric oxide release (64.61%) and reactive oxidation species generation on the pre-treated cells as compared to stressed cells; also, phenols and terpenoids have the ability to inhibit 5-LOX (Albanoa and Miguel, 2011; Amadi et al., 2016).

3.3. Optimization process

3.3.1. Preliminary tests results

Formulation 95/5 was the one chosen according to the results obtained (TPP: 21.92 ± 0.07 mg GAE/g dw; TAC: 37.17 ± 0.11 mg TE/g dw). After centrifugation (5000 rpm/15 min), the first three ratios did not give sufficient extracts for further analysis. Therefore, 1/4 was the ratio used during the test. Roasting the mix of fruits (95/5: *T. tetraptera*/ *A. citratum*) at different temperatures (100–240 °C) revealed a significant ($p < 0.05$) increase in TPP up to 140 °C, followed by a significant decrease at 160 °C when roasting and brewing times were fixed (5 min). Astill et al. (2001) reported that brewing habits vary considerably between countries and among individuals within countries, with brewing times from less than 30 s to 5 min being commonly observed. At a roasting temperature of 140 °C, with a fixed brewing time (5 min), there was an increase in TPP contents when rising the roasting time to 10 min. At a roasting time of 15 min, there was a significant decrease in TPP content. Multiple brewing times were also investigated at a fixed roasting temperature and time (140 °C/10 min). The results show that TPP content increases when the brewing time is raised to 12 min, yet the TPP content decreases significantly after a brewing time of 16 min.

3.3.2. Model fitness and adequacy

The response values (observed and predicted ones) for the different trial combinations and levels of independent process variables (X_1 , X_2 , X_3) in their coded and actual forms are shown in Table 4. There were significant ($p < 0.05$) differences between the TPP content ($25.90 \pm 0.12 - 39.50 \pm 0.16$ mg GAE/g dw) and the TAC ($41.49 \pm 0.27 - 51.01 \pm 0.20$ mgTE/g dw) of extracts found through the different trials.

To verify the adequacy of models, two different tests were used, namely the sequential model sum of squares and model summary statistics. ANOVA showed that the selected quadratic models adequately represented the data obtained for the two responses. The regression models were highly significant ($df: 9$; F -value: 80.78; $p < 0.0001$ for TPP and $df: 9$; F -value: 266.05; $p < 0.0001$ for TAC) and linear ($df: 3$; F -value: 5.68; $p < 0.02$ for TPP and $df: 3$; F -value: 6.55; $p < 0.02$ for TAC), quadratic ($df: 3$; F -value: 229.76; $p < 0.0001$ for TPP and $df: 3$; F -value: 750.99; $p < 0.0001$ for TAC) and interaction ($df: 3$; F -value: 6.91; $p < 0.01$ for TPP and $df: 3$; F -value: 40.62; $p < 0.0001$ for TAC) parameters were significant for the two responses. The empirical relationship between the exper-

imental results found on the basis of CCD and the input variables were expressed by a second-order polynomial equation with interaction terms. Fitting of the data to various models (linear, interactive, quadratic models) was carried out to obtain the regression equations below in terms of coded factors:

$$\begin{aligned} \text{TPP (mg GAE/gdw)} = & 38.80 + 0.20X_1 + 0.97X_2 + 1.02X_3 \\ & - 12.47X_1^2 - 8.87X_2^2 - 4.27X_3^2 \\ & - 3.31X_1X_2 - 0.42X_1X_3 + 0.85X_2X_3 \quad (6) \end{aligned}$$

$$\begin{aligned} \text{TAC (mg TE/gdw)} = & 50.73 + 0.21X_1 + 0.24X_2 + 0.55X_3 \\ & - 8.84X_1^2 - 6.54X_2^2 - 5.31X_3^2 \\ & - 3.43X_1X_2 - 0.16X_1X_3 + 0.67X_2X_3 \quad (7) \end{aligned}$$

The different coefficients of determination (R^2) were 0.98 and 0.99 for TPP and TAC, respectively, implying that 98% and 99% of results, respectively, are based on the quadratic polynomial models obtained. Therefore, the models are sufficient to represent the actual relationship between responses and independent variables. A high R^2 coefficient ensures a satisfactory adjustment of the model to the experimental data (Schubert et al., 2010). The adjusted R^2 values and predicted R^2 values were comparable to the actual R^2 values, suggesting that the fitted models provided an appropriate estimate of the true process. Diagnostic plots such as the predicted versus experimental values (Fig. 1) help us to assess the model satisfactoriness. In this figure, the data points lie reasonably close to the straight line and indicate adequate agreement between the real data and the data obtained from the models. The result suggests that the models used in this research were able to identify effective conditions for TPP extraction and TAC expression from the beverage. Furthermore, correlation analysis was performed and a strong positive correlation was seen between the TPP and the beverage TAC ($p < 0.0001$, $R^2 = 0.93$), as shown in Fig. 2.

All of the models have a non-significant lack of fit ($df: 5$; F -value: 0.95; $p = 0.520$ for TPP $p > 0.05$ and $df: 5$; F -value: 0.96; $p = 0.517$ for TAC), showing the validity of the model according to Malcolmson et al. (1993). Other measures including bias factor ($B_f = 1.000068$ for TPP and 1.000011 for TAC) and accuracy factor ($A_f = 1.000032$ for TPP and 1.00034 for TAC), judged by nearness to unity (1) and not excluding AAD ($AAD = 0.00014$ for TPP and 0.000022 for TAC) values close to zero, all gave suitable results between the predicted and actual data. Indeed, a perfect model is considered when $AAD = 0$; $B_f = 1$ and $A_f = 1$ (Schubert et al., 2010). The ranges of these values obtained in this study show the adequacy of the models for describing the examined parameters.

In addition, the corresponding coefficients of multiple regressions are shown in Table 5. When the TPP of the beverage was studied, the quadratic effects of roasting temperature (X_1), roasting time (X_2), brewing time (X_3), the interaction between roasting temperature and roasting time (X_1X_2) and the linear effects of the brewing time (X_3) and roasting time (X_2) were shown to significantly ($p < 0.05$) influence TPP content. However, these effects (except the linear ones) have a negative impact on the TPP content, meaning that the increase in those variables will reduce the response. The brewing time with an effect of 1.022 was determined to be the most significant independent variable, followed by roasting time (0.972).

When considering the study of the TAC beverage, the linear effect of brewing time (X_3), the interaction between roasting temperature and roasting time (X_1X_2) and the quadratic effects of all independent variables significantly ($p < 0.05$) influence the TAC. The linear effect of the brewing time has a positive impact on the TAC of the beverage, in contrast to the different quadratic effects, and the interaction between roasting temperature and

Table 4

Experimental design for Total Polyphenols (TPP) and Total Antioxidant Capacity (TAC) of the beverage using RSM (coded, actual values and predicted responses).

Run number	Type	Roasting temperature (°C)	Roasting time (min)	Brewing Time (min)	TPP Observed (mg GAE/g dw)	TPP Predicted (mg GAE/g dw)	TAC Observed (mg TE/g dw)	TAC Predicted (mg TE/g dw)
1	Factorial	-1 (130)	1 (15)	+1 (15)	32.51 ± 0.24 ^{de}	32.42	45.27 ± 0.01 ^d	45.27
2		-1 (130)	-1 (10)	-1 (8)	27.40 ± 0.10 ^{fg}	27.40	41.73 ± 0.22 ^{ij}	41.78
3		-1 (130)	-1 (10)	+1 (15)	28.42 ± 0.20 ^{efg}	28.31	42.14 ± 0.21 ⁱ	42.07
4		1 (170)	-1 (10)	+1 (15)	30.30 ± 0.04 ^{def}	30.61	44.39 ± 0.14 ^{efg}	44.65
5		-1 (130)	+1 (15)	-1 (8)	30.13 ± 0.07 ^{defg}	30.30	44.41 ± 0.21 ^{efg}	44.02
6		+1 (170)	+1 (15)	1 (15)	29.50 ± 0.16 ^{efg}	30.02	43.16 ± 0.06 ^h	42.98
7		+1 (170)	+1 (15)	-1 (8)	27.91 ± 0.10 ^{fg}	28.50	42.02 ± 0.12 ⁱ	41.96
8		+1 (170)	-1 (10)	-1 (8)	29.75 ± 0.17 ^{efg}	30.30	44.71 ± 0.16 ^e	44.58
9	Centre	0 (150)	0 (12.5)	0 (11.5)	38.50 ± 0.08 ^a	38.80	50.14 ± 0.10 ^b	50.73
10		0 (150)	0 (12.5)	0 (11.5)	38.81 ± 0.10 ^a	38.80	50.66 ± 0.12 ^{ab}	50.73
11		0 (150)	0 (12.5)	0 (11.5)	38.47 ± 0.10 ^a	38.80	51.01 ± 0.20 ^a	50.73
12		0 (150)	0 (12.5)	0 (11.5)	39.50 ± 0.16 ^a	38.80	50.94 ± 0.17 ^a	50.73
13		0 (150)	0 (12.5)	0 (11.5)	37.71 ± 0.16 ^{ab}	38.80	50.93 ± 0.12 ^a	50.73
14		0 (150)	0 (12.5)	0 (11.5)	39.80 ± 0.12 ^a	38.80	50.75 ± 0.05 ^a	50.73
15	Axial	+1.68 (183.6)	0 (12.5)	0 (11.5)	27.25 ± 0.10 ^{fg}	26.53	42.09 ± 0.26 ⁱ	42.10
16		-1.68 (116.4)	0 (12.5)	0 (11.5)	25.9 ± 0.12 ^g	26.12	41.49 ± 0.27 ^j	41.67
17		0 (150)	+1.68 (16.7)	0 (11.5)	31.43 ± 0.14 ^{def}	30.90	44.12 ± 0.20 ^{fg}	44.43
18		0 (150)	-1.68 (8.3)	0 (11.5)	29.21 ± 0.20 ^{efg}	28.85	44.07 ± 0.10 ^g	43.95
19		0 (150)	0 (12.5)	+1.68 (17.4)	35.68 ± 0.11 ^{abc}	35.55	44.61 ± 0.24 ^{ef}	44.87
20		0 (150)	0 (12.5)	-1.68 (5.6)	34.11 ± 0.10 ^{bcd}	33.50	46.04 ± 0.09 ^c	45.97

Means ± standard deviations of three observations followed by different superscripts (a–j) are significantly different: GAE: Gallic acid equivalent; TE: Trolox Equivalent; dw: dry weight.

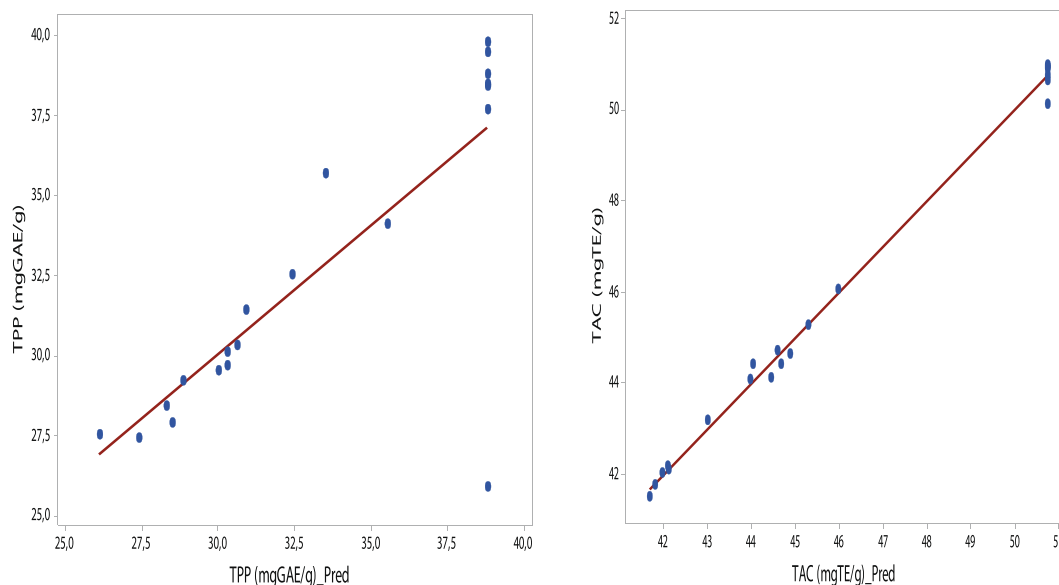


Fig. 1. Comparison between predicted and experimental values of Total polyphenols (TPP) and Total antioxidant capacity of the beverage.

roasting time. Brewing time, with an effect of 0.329, was the most significant independent variable, followed by roasting time (0.144).

3.3.3. Effects of the extraction conditions on TPP content and the beverage TAC and optimum extraction conditions

Solubility of phenolic compounds is governed by their chemical nature in the plant that may vary from simple to very highly polymerized substances. The solubility is also affected by the polarity of the used solvent(s), the type of plant materials, and variable processes that plants undergo during food transformation. In order to obtain highest TPP and TAC, it is important to find the best roasting temperature-roasting time-brewing time ratio.

Fig. 3 shows the effect of the interaction of the various independent factors on the TPP content and TAC potential of the beverage.

The TPP content and the TAC increase with increasing roasting temperature, roasting time and brewing to a certain extent, and then changed slightly. In fact, the TPP of the beverage increased until the midpoint of the response surface was reached. However, further increases in independent variables slightly reduced the TPP. As shown in Fig. 3(a and b), TPP and TAC are function of roasting temperature, roasting time at fixed brewing time (11.5). It is shown that roasting time had high influence with positive effect on yields of both responses. Fig. 3(c and d) show the effect of roasting temperature and brewing time on the two responses at fixed roasting time (12.5 min). The yields of TPP and TAC increase with the increase of the roasting temperature (between 140 °C and 160 °C) and the brewing time (10 and 14 min). Fig. 3(e and f) present the effect of brewing time and roasting time on TPP and TAC at fixed roasting temperature (150 °C). For Fig. 3(c–f) the brewing

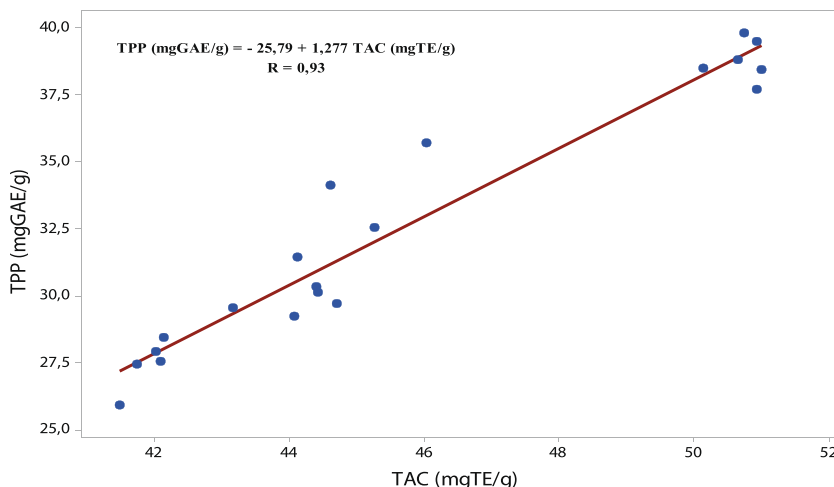


Fig. 2. Correlation between total polyphenols (TPP) content and total antioxidant capacity (TAC) of the beverage.

Table 5

Regression coefficients of predicted quadratic polynomial models for the two responses.

Terms	Total polyphenols (mg GAE/g dw)				Total antioxidant Capacity (mg TE/g dw)			
	Estimation	SE	T ratio	P-Value	Estimation	SE	T ratio	P-Value
b_0	38.804	0.310	125.38	0.000*	50.732	0.129	391.71	<0.0001*
X_1	0.208	0.345	0.60	0.560	0.127	0.085	1.48	0.1692
X_2	0.972	0.345	2.81	0.018*	0.144	0.085	1.68	0.1234
X_3	1.022	0.346	2.96	0.014*	0.329	0.085	3.83	0.0033*
$X_1 X_2$	-3.316	0.757	-4.38	0.001*	-1.216	0.112	-10.83	<0.0001*
$X_1 X_3$	-0.425	0.760	-0.56	0.588	-0.056	0.112	-0.50	0.6272
$X_2 X_3$	0.850	0.760	1.12	0.290	0.238	0.112	2.13	0.0594
X_1^2	-12.475	0.565	-22.07	0.000*	-3.128	0.083	-37.40	<0.0001*
X_2^2	-8.875	0.565	-15.70	0.000*	-2.313	0.083	-27.66	<0.0001*
X_3^2	-4.272	0.566	-7.55	0.000*	-1.878	0.083	-22.46	<0.0001*

SE: Standard Error; X_1 : Roasting temperature ($^{\circ}$ C); X_2 : Roasting time (min); X_3 : Brewing time (min); GAE: Gallic acid Equivalent; TE: Trolox Equivalent.

* $p < 0.05$.

time had the highest influence with the positive effect on TPP and extracts TAC.

The highest TPP (38.90 mg GAE/g dw) content in the beverage was obtained for a roasting temperature of 150 $^{\circ}$ C, a roasting time of 12.8 min and a brewing time of 12.3 min, with a desirability of 0.94. Concerning the TAC of the beverage, as described earlier, the response increased when the independent variables increased. However, the maximum TAC (50.75 mg ET/g dw) of the beverage is obtained at 150.34 $^{\circ}$ C for 12.54 min (roasting time) for a brewing time of 11.81 min, with a desirability of 0.97. After 13.5 min of roasting and brewing times and a temperature of around 157 $^{\circ}$ C, there was a significant decrease in the beverage TAC. Considering the significant interaction between roasting temperature (X_1) and roasting time (X_2) in Table 5, it can be said that the roasting temperature did not have the same effect on the TPP and TAC of the beverage when the roasting time was changed.

When various responses have to be considered at the same time, it is necessary to identify optimal compromises between the total numbers of responses taken into account. Therefore, the predicted optimal conditions to obtain maximum TPP content and TAC of the beverage are a roasting temperature of 150 $^{\circ}$ C during 12.62 min and a brewing time of 11.91 min, with a desirability of 0.95. The optimum conditions obtained in this case are different to those recorded by Zzaman et al. (2014) after roasting cocoa beans. In that study, numerical optimisation and superimposed contour plots suggested the optimal roasting conditions to be 192 $^{\circ}$ C with a roasting time of 10 min; these conditions for roasting

cocoa beans produce high-quality cocoa products in terms of antioxidant properties.

The increased extractability of phenolic compounds might be attributed to the increase in dependent variables by the disruption of plant cell walls through high heat treatment; this might cause phenolic compounds to be released more easily than in the raw materials (Pérez-Burillo et al., 2018). As the Folin-Ciocalteu method is based on the reducing power of the hydroxyl group, the greater the level of phenolic hydroxyl groups, the higher the level of TPP assayed using the Folin-Ciocalteu method. In addition, our results record a significant correlation between TPP content and the antioxidant activity of the beverage. The increase in TPP content leads to an increase in the beverage TAC, as previously mentioned by Sokamte et al. (2019). The increase in antioxidant capacity can be linked to the better release of phenolic acids, flavonols, the degradation of tannins to simple phenolics, and the contribution of Maillard reaction products following roasting processes (Pokorný and Schmidt, 2010). According to previous groups, during Maillard reactions, compounds with free amino groups (lysine) can undergo a sequence of complex reactions with carbonyl compounds to produce products such as furans (tetrahydrofuran), melanoidins, pyrroles, pyrazines, and other heterocyclic compounds that contribute to enhanced antioxidant activity. As *T. tetraptera* fruits contain a large amount of carbohydrates (Akintola et al., 2016) and amino acids (Bouba et al., 2016), we believe that Maillard reaction-derived products might be non-negligible contributors to enhancing the antioxidant attributes of spices during roasting. In addition to the heat impact, a general increase in influ-

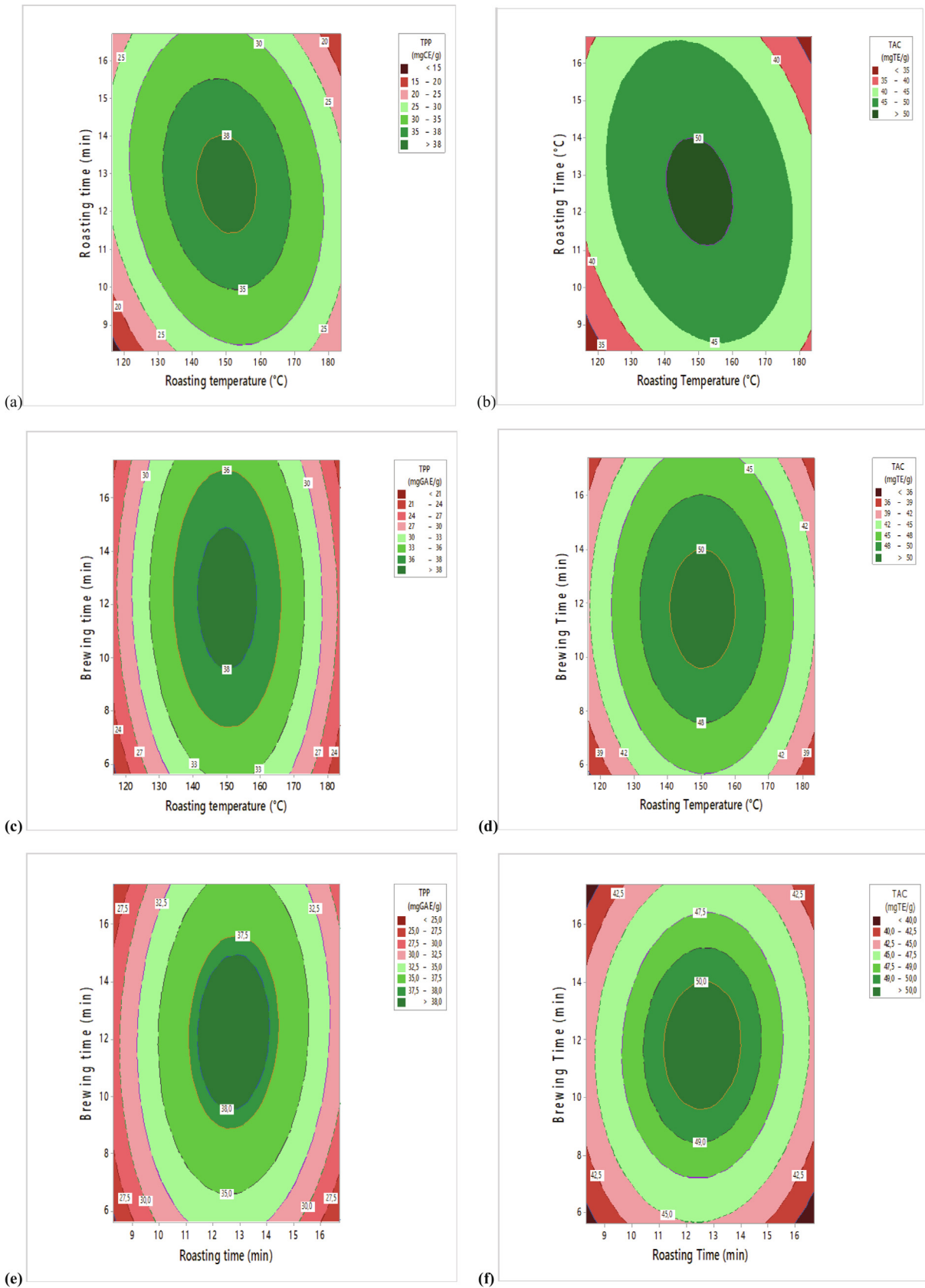


Fig. 3. Contour plots for the effects of independent variables: Roasting temperature, Roasting time and brewing time on the TPP (mg GAE/g dw) and TAC (mg TE/g dw) of the beverage.

sion time amounted to the extraction of more bioactive compounds (Pérez-Burillo et al., 2018). However, as shown in Fig. 3, TPP content and TAC of the beverage increased with higher temperatures and longer steeping times; if lower temperatures were to be used, extended steeping times were required. These results are in accordance with a previous study using *Centella asiatica* tea (Siah et al., 2011). It is possible that the concentration of phenolic components may increase up to certain temperatures due to favourable reactions, leading to their higher recovery into the solvents.

However, prolonged heating may decrease the concentration of naturally occurring polyphenolics in food products (Ioannou and Ghoul, 2012). It was found that increased heating times reduced flavonoids and TPP. This means that the active compounds were heat labile or easily destroyed (Memnune et al., 2014). The decrease in phenolic compounds can also be explained by the high redox activity of polyphenols at those conditions (Wollgast and Anklam, 2000). The same results were observed in tea infused at 100 °C, where steeping time beyond 5 min caused a decrease in TPP. Cheong et al. (2005) and Perva et al. (2006); Cited by Siah et al. (2011) showed that prolonged infusion times at high temperatures can lead to the degradation of phenolic compounds.

The lack of tests or data on the roasting effects on *T. tetraptera* and *A. citratum* fruits phytochemistry limits the comparison of the results. However, the results obtained by Nwaichi and Anyanwu (2013) indicate that for an exposure to heat (100 °C) for 0, 30, 60, and 90 min, the authors registered an increase in TPP up to 30 min and a decrease at 60 and 90 min in *T. tetraptera* aqueous extracts compared to unheated fruits. In contrast, after 30 min, flavonoid concentrations and the antioxidant activity of extracts increased with elevated heat. Similar observations were made for common spices after heat treatment. Romson et al. (2011) reported that heat treatment increased TPC and the antioxidant activity of turmeric-chili paste and its ingredients. They suggested that bound phenolic and flavonoid compounds could be liberated by heat treatment. (Nikousaleh and Prakash, 2016) reported that clove exhibited a powerful antioxidant activity, which increased in dry heat treatments and correlated positively with antioxidant components.

3.4. Verification of the optimised conditions and predictive model

The suitability of the model equations for predicting optimum response values was tested under the following conditions: roasting temperature, 150 °C; roasting time, 12.62 min and brewing time, 11.91 min. The experiments were carried out in triplicate to compare the experimental results with the predicted values of the responses using polynomial equations. The experimental values are reported in Table 6. The mean values of the responses registered are not far from the predicted values and indicate the suitability of the developed quadratic models. The percentage deviation of the experimental and predicted values was found to be 0.72% and 0.65%, respectively, for TPP content and TAC. These responses are within 95% of the predicted values and indicate the suitability of the developed quadratic models.

4. Conclusion

The two spices (*Aframomum citratum* and *Tetrapleura tetraptera* fruits) are good source of phenolic compounds. The study of some biological *in vitro* activities reveals their dose dependent antioxidant and anti-inflammatory abilities. The main goal of this research was to find the best settings for phenolic compounds extraction (the most optimal roasting temperature, roasting time and brewing time). Desirability function was developed for the following criteria: maximum content of total polyphenols (TPP) and maximum total antioxidant capacity (TAC) of the roasted mixed (*T. tetraptera/A. citratum*: 95/5) aqueous extracts.

Mathematical models were suggested for the two responses (TPP and TAC) and the data fit well with an R^2 of 98.64% and 99.58%, respectively, for TPP content and TAC. The most influencing parameter is the brewing time. By applying the desirability function method, the optimum extractions conditions obtained are: roasting temperature of 150 °C, roasting time of 12.62 min and brewing time of 11.91 min. Additional settings were offered by the software to achieve 38.87 mg GAE/g dw of TPP and 50.75 mg TE/g dw TAC. High correlation of the mathematical model indicated that a quadratic polynomial model could be employed to optimize the solid–liquid extraction of antioxidants from these fruits. Under optimal conditions, the experimental values were in agreement with the predicted values. A focus for further studies would be monitoring the levels of predominant, nutritional and therapeutic components during the process to determine whether or not the process affects their content. Indeed, each bioactive compound possesses specific extraction conditions from which its release in the solvent can be optimised. A further important focus must be to study the correlation between the optimum extraction process and the organoleptic characteristics of the beverage made with the two spices because the extracted molecules generally affect the taste of the infusion.

5. Public interest statement

Tetrapleura tetraptera and *Aframomum* spp. fruits are Non-Timber Forest Products spices commonly used in sub-Saharan Africa especially in Cameroon for many purposes: flavor enhancers and therapeutic issues. They have been identified among spices commonly used and available in cities and are components of traditional meals and daily diets. Many studies have been documented and revealed their usefulness in food intake by their physicochemical content. They are also successfully introduced in several foodstuffs for preservation and to ameliorate sensory properties. They contain chemical compounds that confer to them many interesting biological activities. All those bioactive compounds can undergo denaturation when the extraction process is not controlled or not well mastered. Through this novel study, we are defining parameters than can affect the extraction yield of polyphenols and the ranges or values to apply in order to get the optimal content in polyphenols corresponding to the highest total antioxidant capacity of a beverage made with the mix of the spices on a home food consumption context.

Table 6

Predicted and experimental values of the dependent variables at optimum conditions.

Optimal level of process parameters	Optimized values (predicted values)		Experimental values	
Roasting temperature: 150 °C	TPP (mg GAE/g dw)	TAC (mg TE/g dw)	TPP (mg GAE/g dw)	TAC (mg TE/g dw)
Roasting time: 12.62 min	38.87	50.75	38.59 ± 0.11	50.42 ± 0.14
Brewing time: 11.91 min				

TPP: Total Polyphenols; TAC: Total Antioxidant Capacity; GAE: Gallic acid equivalent; TE: Trolox Equivalent; dw: dry weight.

6. Funder information

The authors received no funding for this research.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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