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Optimization of green extraction of phenol and flavonoid from *Glycine max* seeds by the Microwave Assisted Extraction (MAE) method.

Optimisation de l'extraction des composés phénoliques de la graine de Glycine max par la méthode d'extraction assistée par microonde.

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ABSTRACT:

The present study aimed at determining the optimal conditions for extraction of polyphenolic and flavonoid compounds from soybean seeds using a green protocol with Microwave Assisted Extraction (MAE). A Face Center Composite Design (FCCD) was used for optimization. A 50 % hydro-ethanolic solution was used with solvent/dry matter ratio (60/1 - 110/1), wavelength (120 - 270 W) and time (0 - 10 min) as factors while the responses studied were polyphenolic and flavonoid contents. The factors that significantly influenced both responses were individual effect of all factors, interaction between solvent/dry matter ratio and extraction time, quadratic effect of solvent/dry matter ratio and wavelength for polyphenolic content, while only quadratic effect of wavelength significantly influenced the flavonoid content. Highest contents of phenols (6.87 mg GAE/g) and flavonoid (4.46 mg CE/g) were obtained at 150 W for 2 min with a solvent ratio of 70:1. RSM permitted us to develop a green protocol for maximum extraction of phenols and flavonoid using less solvent, low wavelength and a reduced time in MAE.

Keywords: Optimization, Phenol, Flavonoid, Soybean, Microwave Assisted Extraction

RÉSUMÉ :

Le présent travail avait pour but de déterminer les conditions optimales d'extraction des phénols totaux et des flavonoïdes des grains de *Glycine max* (soja) par la méthode d'extraction assistée par microonde. Le plan composite centré a été utilisé avec pour facteurs le rapport solvant/matière sèche (60/1 - 110/1), la longueur d'onde du microonde (120 - 270 W) et le temps d'extraction (0 - 10 min), tandis que les réponses étudiées étaient les teneurs en phénols totaux et en flavonoïdes. Le solvant d'extraction était une solution hydro-éthanolique (50 %). Tous les facteurs pris individuellement ont significativement influencés les deux réponses, de même que l'interaction entre le rapport solvant/matière sèche et le temps d'extraction, l'effet quadratique du rapport solvant/matière sèche et de la longueur d'onde pour les phénols totaux, tandis que seul l'effet quadratique de la longueur d'onde a montré une influence significative sur la teneur en flavonoïdes. Les teneurs maximales en phénols (6,87 mg GAE/g) et flavonoïdes (4,46 mg CE/g) ont été obtenues à 150 W pendant 2 min un rapport solvant/matière sèche de 70/1. Le plan de surface réponse nous a permis de développer un protocole ''vert'' pour une extraction optimale de phénols totaux et des flavonoïdes à partir des graines de soja avec peu de solvant, à une faible longueur d'onde et en un temps réduit par la méthode d'extraction assistée par microonde.

Mots clés : Optimisation, Phénols, Flavonoïdes, Soja, Extraction assistée par microonde.

1. INTRODUCTION

Glycine max (soybean) seeds have long been reported as one of the richest flavonoid sources known nowadays; with up to 3 mg/g dry weight (Ajay et al., 2011; Babu et al., 2011). Flavonoid,as other phenolic compounds are known to have antioxidants, anti-inflammatory, anti-cancer activities among other (Sharma et Beluja, 2015). Common methods such as traditional maceration, soxlhet are often used to extract phenol and flavonoid from leaves, seeds, bark and other parts. Recently the use of super critical fluid, Ultrasound Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE) methods for extraction of phenols are been developped (Ali et al., 2013; Shadab, 2019). Nonetheless, UAE and MAE are proven to be the most effective in terms of phenol and flavonoid yield, greener, respectful to the environment (Mandal et al., 2007; Felipe, 2020). But trials to extract these bioactive compounds from soybean seeds have lead to astonishingly disparate results: extraction yield and even the quality of the molecules obtained are dependent on many factors including solvent type and proportion, extraction method, and time. As a result, scientists started using very toxic solvent, high temperature and more energy to maximize the extraction yield of phenols and flavonoid from soybean seeds, what unfortunately contributed enough in pollution of our planet. Trying to reverse or at least limit the damages, exigencies of the so called green chemistry nowadays appeal researchers to use ‘‘green’’ processes in science. Microwave is the most accessible recent extraction technology compared to ultrasound or supercritical fluid, thus explains our preference using it. It is noticed from the literature that yields of modern extraction techniques likeUAE and MAE are conditioned by many factors including nature of the extracting solvent, time of extraction, power of the equipment, dry matter/solventratio and the nature of the matrix (Mandal et*al.*, 2007). So, determination of experimental conditions for extraction of the highest phenol and flavonoid content from soybean seeds using a green protocol is quite urgent since these seeds are among the richest sources of the named compounds. This study aimed at determining the optimal conditions for extraction of polyphenols and flavonoid using MAE.

2. MATERIAL AND METHODS

2.1. Material

Seeds of *Glycine max* (variety*TGX-1850-10E*) were purchased from “Institut de Recherche Agricole pour le developpement” (IRAD) Foumbot, West Cameroon and sorted.

2.2. Method

2.2.1. Preparation of sample

Seeds of *Glycine max* were dried in an oven at 45 °C until constant weight, and then finely ground using an electrical grinder (royalty line, 800 W, five cycles of 1 min each at full power). Powders were then sieved (using a 500 µm sieve) and immediately used for extraction of phenols and flavonoid.

2.2.2. Screening of factors affecting the phenol and flavonoid contents

On the basis of the literature, variables retained for screening were: time of extraction, dry matter/solvent ratio, proportion of ethanol, and wavelength. Factors influencing the yields of phenols and flavonoids were determined from table 1:

Table 1: Experimental tests.

Tested factor	Experiment	1	2	3
Wavelength				
	Wavelength(W)	120	180	240
	Time (min)	5	5	5
	Ethanol (%)	20	20	20
	Solvent (mL)	30	30	30
Time (min)				
	Wavelength(W)	240	240	240
	Time (min)	2	4	6
	Ethanol (%)	20	20	20
	Solvent (mL)	30	30	30
Solvent Proportion				
	Wavelength(W)	240	240	240
	Time (min)	5	5	5
	Ethanol (%)	10	20	30
	Solvent (mL)	30	30	30
Dry matter/solvent ratio				
	Wavelength(W)	240	240	240
	Time (min)	5	5	5
	Ethanol (%)	20	20	20
	Solvent (mL)	20	30	40

2.2.3. Extraction of phenol and flavonoid

For each trial, 1g of seeds' powder was mixed with the appropriate amount of solvent according to the experiment conditions as given by the chosen design. The mixture was stirred using a magnetic agitator, afterward, it was allowed to rest for 10 minutes at room temperature before being put in the indicated condition for extraction. The supernatant was collected after extraction by filtering through Watman paper n°4. Solvent was then evaporated in an air oven at 45 °C for 24 hours. Dry extracts were immediately used for determination of phenols and flavonoid content.

2.2.4. Determination of responses

Determination of total phenolic content

The total phenolic content was assessed according to the method proposed by Dohou et al. (2003). Briefly, 0.2 mL of Folin reagent (tenfold diluted) was added to a tube containing 0.01 mL of plant extract (5 mg/mL) and 1.39 mL of distilled water. The mixture was allowed to stand for 3 minutes before addition of 0.4 mL of Sodium carbonate (20 % w/v), and then mixed using a vortex. The tube was then incubated at 40 °C for 20 min in a water bath and absorbance was read at 760 nm against a blank using a BIOMATE spectrophotometer. Gallic acid (0.2 g/L) was used to draw a calibration curve. All experiments were carried out in triplicate and results were expressed as mg of gallic acid equivalent (GAE) per g of dry extract (mg GAE/g dry weight).

Determination of flavonoid content

Flavonoid content was obtained using the method described by Padmadja et al. (2011). 0.03 mL of sodium nitrite (5 %) wasadded to a tube containing 1.49 mL of water and 0.1 mL of extract solution (5 g/mL). After 5 min, a volume (0.003 mL) of aluminium chloride (10 %) was added to the tube and the mixture was allowedto rest for 6 min.Afterward, 0.3 mL of NaOH (1M) and 0.24 mL of water was introduced respectively in the tube and mixed with a vortex before absorbance was read at 510 nm against a blank. Calibration curved was made using catechin. All experiments were made in triplicate and results expressed as mg of catechin equivalent per g of dry extract (mg CE/g of dry weight).

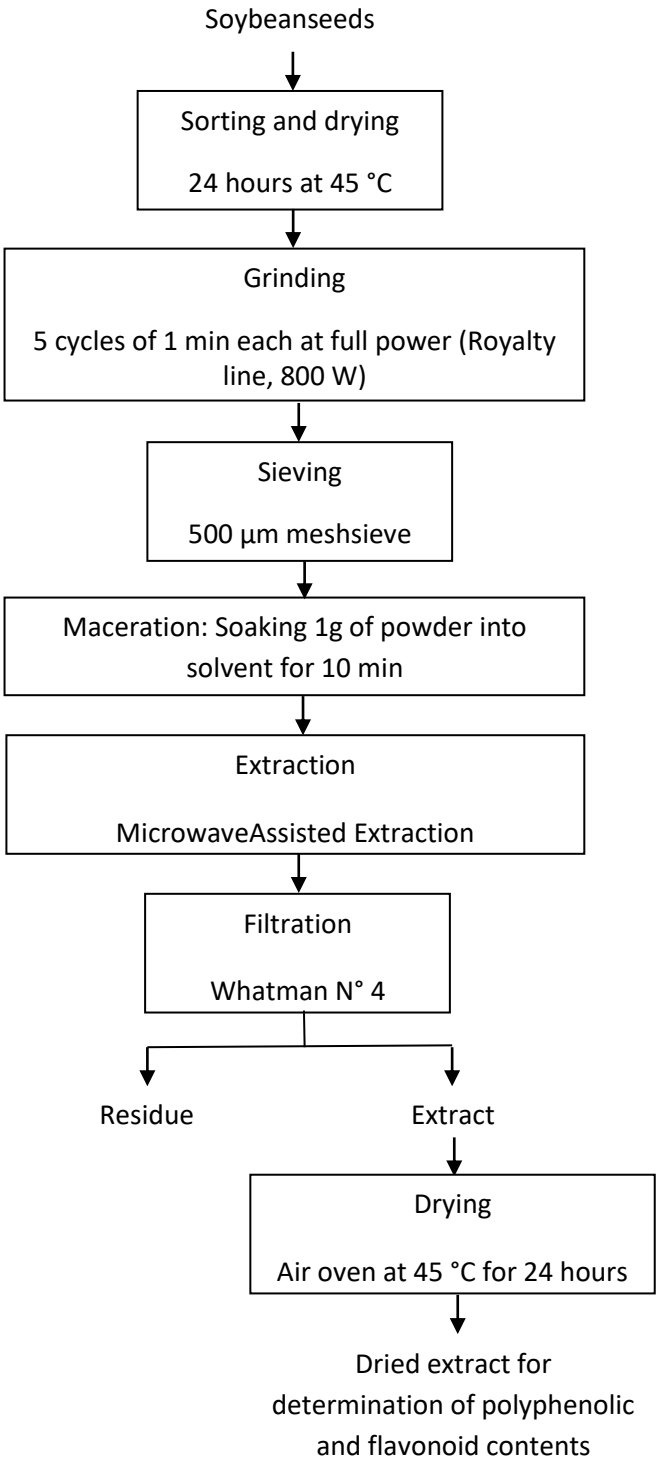


Figure 1: Extraction flowchart of phenols and flavonoid from soybean seeds

2.2.5. Optimization of the responses using the Central composite design

All factors: time of extraction, dry matter/solvent ratio and wavelength were observed to influence the responses. We used the center composite design and the studied responses were total phenolic content (Y_1) and flavonoid content (Y_2). Ranges of different factors were taken according to the results of preliminary experiments. Experiments were randomized and carried out in triplicate. The proposed models were:

$$Y = a_0+a_1X_1+a_2X_2+a_3X_3+a_{11}X_1^2+a_{22}X_2^2+a_{33}X_3^2+a_{12}X_1X_2+a_{13}X_1X_3+a_{23}X_2X_3$$

Where Y is the response (phenols or flavonoid content), X_1, X_2, X_3 are the studied factors, a_0 is the offset term while a_1, a_2, $a_3$are linear effects, a_{11}, a_{22}, $a_{33}$the quadratic effects and a_{12}, a_{13}, a_{14}, a_{23}, a_{34} are interaction effects.

Verification of the model and optimum condition

Table 2: matrix of coded and real variables with responses obtained according to experimental conditions and predicted values

Trials	Matrix of real and coded variables			Responses			
				Polyphenolic (mg GAE/g)		Flavonoid (mg CE/g)	
	Solvent	Wavelenght	Time	Exp	Pre	Exp	Pre
1	70.00(-1)	150.00(-1)	2.00(-1)	6.87±0.14	6.94	4.46±0.05	4.25
2	100.00(1)	150.00(-1)	2.00(-1)	3.66±0.10	3.87	3.31±0.07	3.20
3	70.00(-1)	240.00(1)	2.00(-1)	4.87±0.09	5.10	2.34±0.02	2.61
4	100.00(1)	240.00(1)	2.00(-1)	1.96±0.17	1.67	1.43±0.05	1.29
5	70.00(-1)	150.00(-1)	8.00(1)	2.09±0.15	2.83	1.25±0.15	1.45
6	100.00(1)	150.00(-1)	8.00(1)	4.24±0.18	4.46	2.11±0.05	1.91
7	70.00(-1)	240.00(1)	8.00(1)	1.75±0.02	1.99	1.70±0.07	1.88
8	100.00(1)	240.00(1)	8.00(1)	2.89±0.09	3.26	1.79±0.05	2.07
9	59.77(-1,68)	195.00(0)	5.00(0)	6.21±0.11	5.66	2.65±0.02	2.40
10	110.22(1.68)	195.00(0)	5.00(0)	4.24±0.06	4.14	1.55±0.07	1.67
11	85.00(0)	119.31(-1.68)	5.00(0)	5.80±0.14	5.27	3.21±0.10	3.42
12	85.00(0)	270.68(1.68)	5.00(0)	2.83±0.11	2.71	2.51±0.10	2.18
13	85.00(0)	195.00(0)	-0.04(-1.68)	3.15±0.02	3.23	2.80±0.02	2.93
14	85.00(0)	195.00(0)	10.04(1.68)	1.84±0.09	1.11	1.48±0.07	1.23
15	**85.00(0)**	**195.00(0)**	**5.00(0)**	2.54±0.04	2.43	1.85±0.02	1.85
16	**85.00(0)**	**195.00(0)**	**5.00(0)**	2.42±0.05	2.43	1.92±0.05	1.85
17	**85.00(0)**	**195.00(0)**	**5.00(0)**	2.51±0.15	2.43	1.84±0.02	1.85
18	**85.00(0)**	**195.00(0)**	**5.00(0)**	2.30±0.05	2.43	1.84±0.02	1.85
19	**85.00(0)**	**195.00(0)**	**5.00(0)**	2.33±0.06	2.43	1.82±0.05	1.85
20	**85.00(0)**	**195.00(0)**	**5.00(0)**	2.39±0.06	2.43	1.84±0.02	1.85

Bold values are replicates of the center points. Pre are predicted values

2.2.6. Statistical analysis

Designing and analysis of the results were done using Minitab 18. Experiments were carried out in triplicate. Statistical significance of the model variables was determined at 5 % probability level. Main effects and contour plots were plotted using Sigma Plot v11.0 (c) systat. Data on phenol and flavonoid contents were expressed as mean± SD and compared using Bonferroni test with the software SPSS version 22.

3. RESULTS AND DISCUSSION

3.1. Screening factors

3.1.1. Results of the screening

Results of the screening factors were as indicated in table 3: usage of high wavelength lead to a reduction in both the polyphenolic and the flavonoid content of the extracts. Long cooking time almost induced a linear reduction of the two studiedresponses. Short times were seen to be best for highest responses. Variation of ethanol proportion in the extracting solvent lead to a linear progression of both responses.Thisobservation means ethanol may be more effective in extracting polyphenol and flavonoid than a hydro-ethanolic solvent. Effect of dry matter/solvent ratio: we noticed that any increase in the solvent proportion leads to an increase inboth polyphenolic and flavonoid contents.

Table 3: conditions and responses for the screening

Experiment	Conditions	Phenols (mg GAE/g)	Flavonoids mg CE/g)
Wavelength			
	W (120W)	1.9	1
	W (180W)	1.3	0.8
	W (240W)	0.8	0.4
Time (min)			
	T (2min)	2.9	1.3
	T (5min)	1.5	0.7
	T (8min)	1.3	0.6
Ethanol Proportion			
	E(20 %)	1.9	1.2
	E(50 %)	4.3	2.4
	E(80 %)	2.1	1.3
Dry matter/solvent ratio			
	DM/S (30)	1.2	0.4
	DM/S (60)	3.9	1.3
	DM/S (80)	2.1	1.8

While testing the influence of any factor, the others were respectively kept constant at: wavelength (180 W), time (5 min), solvent /dry matter ratio (30/1) and ethanol proportion (20 %).

3.1.2. Optimization of the responses using the Central composite design

Results of the screening permitted the optimization of the process using three (3) main factors for the production of phenol and flavonoid from soybean seeds, namely the microwave wavelength, the cooking time and the solvent/dry matter ratio. Ethanol proportion was decided to be 50 % since wehad noticed a maximal production with 50 % ethanol proportion (in the solvent) in the phenol and flavonoid contents during the screening. Also, literature indicates that, dielectric properties of the solvent should be highly taken into consideration when planning to extract phenolic compounds using MAE. Compared to water, ethanol or its mixtures with water have a lower dielectric constant, and are more transparent to microwave, thus not well converting them into heat. But its high capacity to dissolve and extract phenolic compounds (Inglett et al., 2010; Rafiee et al., 2011; Naima et al., 2019) and its greenness oriented our choice of an hydro-ethanolic solution as extracting solvent. Table 2 gives the different factors retained for optimization, in coded and real values with experimental and predicted values of the responses.

3.1.3. Analysis of main effects

The entire experimental plan consisted of 20 trials. Responses were evaluated in triplicate. The highest polyphenolic content (6.87 mg of GAE/g of dry seed weight) was obtained at 150 W for 2 min of cooking time with 70 mL of solvent.The lowest content (1.75mg GAE/mg) is observed at 240W of microwave power with 70 mL of extracting solvent and a heating time of 8 min.Concerning the flavonoid content, the highest value (4. 46 mg QE/g of dry weight) was obtained at 150 W of microwave power, 2 min heating time in 30 mL of solvent.The lowest flavonoid content(1.25mg CE/g) is obtained with 70 mL of solvent at 150 W for a boiling time of 2 min. values ranging from 1.75 to 6.78 mg of GAE/g and 1.25 CE/g to 4.46 CE/g of extract for polyphenolic and flavonoid content respectively were similar to those obtained by Djordje et al. (2007), Sakthivelu et al.(2008) and Ana et al. (2016).

Effect of solvent ratio

Figure1shows that increase in the solvent ratio induces an almost linear reduction the polyphenolic content of extracts when going from 60/1 to 80/1 (mL of solvent/g of seed dry weight) solvent ratio. But the responses measured started increasing as the solvent ratio passed from 90:1 to greater values. Such results indicates that, the researcher is free to choose between less or more solvent to extract phenols from soybean seeds, even if less solvent is advised for a reduction of energy consumption and greenness of the method. Further research isneeded to know the types of phenolic compounds obtained at low and high solvent ratio since literature indicatesthat free or bound phenolic compounds are found in soybean and both are not extracted in the same experimental conditions, solvent ratio/dry matter included (Sanja et al., 2018). Nevertheless, it is well accepted nowadays that high solvent ratio increase mobility of compounds (mass transfer) from plants matrix, thus explaining the observed increase inthe polyphenolic content of extracts at certain solvent ratio, since previous research had already reported that (Mohamed & Chang, 2008). Increase in the solvent ratio only led to a progressive diminution of the flavonoid content.

Effect of wavelength

Figures 2and 3 depict the influence of wavelength on the polyphenolic and flavonoid contents of extracts. We can see from the figures that increase in the wavelength induces a reduction in the phenol and

flavonoid contents of soybean seeds extracts. This is a consequence of degradation of these compounds exposed to high temperature, since high power in microwave induce a quick elevation of the solvent temperature even when exposure is for a short duration. Sanja et al. (2018) made similar observation.

Effect of time

From the following figures, we can see that any increase in time of exposure also led to linear diminution of the phenolic and flavonoid contents of extractsbecauseof progressive destruction of these thermo-sensitive compounds under long exposure to heat. Previous authors also noticed the same effect (Rafiee et al., 2011; Sanja et al., 2018 and Xuan et al., 2019).

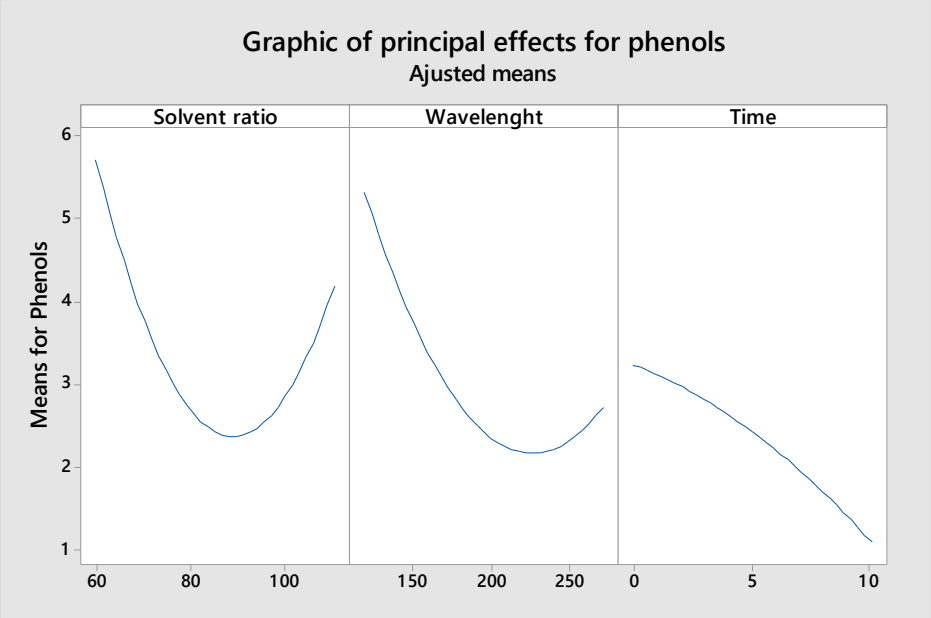


Figure 2: main effect plots of individual factors on polyphenolic content

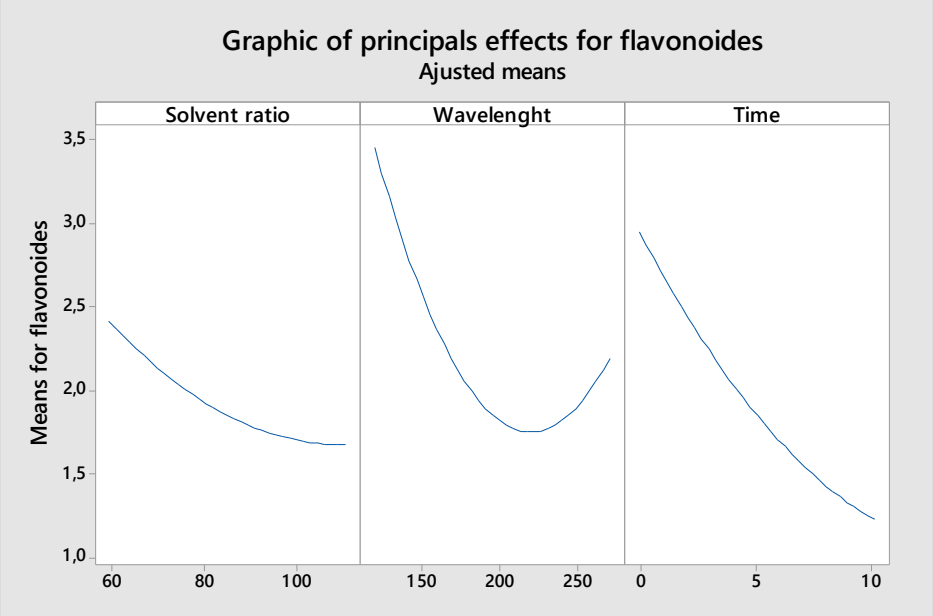


Figure 3: main effect plots of individual factors on flavonoid content

3.1.4. ANOVA, regression equations for the responses

Table 6 shows the ANOVA, the influence of each independent factor. We can see from the table that all independent factors significantly(p <0, 05) influenced both polyphenolic and flavonoid content. Quadratic effects of solvent ratio (X_1* X_1) and thewavelength (X_3* X_3) significantly affected the polyphenolic content of the extract obtained while only the quadratic effect of the heating wavelength (X_3* X_3) significantlyinfluenced the flavonoid content of the extracts. Interaction between solvent ratio and the heating time(X_1X_2) significantly impacted both the polyphenolic and the flavonoid contentwhile only interaction between the time and the boiling microwave wavelength (X_2X_3) significantly affected the flavonoid content of the extracts. We can also see that interaction between solvent ratio and the heating time (X_1X_3) contributed the most in the observed phenolic response (24.74 %), followed by the quadratic effect of the solvent ratio (X_1X_1) which contributed up to 22.17 % to the final response. Talking about the phenolic content, time (X_3) and interaction between wavelength and time (X_2X_3) contributed the most to the observed response (30.06 % and 18.33 respectively).

The mathematical model predicting the influence of the solvent ratio, boiling time and working wavelengthon the phenolic and flavonoid contents of the extracts are given by equations 1 and 2.

$$\text{Phenolic content} = 58.24 - 0.795\,X_1 - 0.1213\,X_2 - 2.693\,X_3 + 0.003883\,X_1X_1 + 0.000273\,X_2X_2 - 0.0102\,X_3X_3 - 0.000131\,X_1X_2 + 0.02614\,X_1X_3 + 0.00186\,X_2X_3 \quad (1)$$

$$\text{Flavonoid} = 19.86 - 0.0876\,X_1 - 0.0836\,X_2 - 1.715\,X_3 + 0.000297\,X_1X_1 + 0.000166\,X_2X_2 + 0.00900\,X_3X_3 - 0.000098\,X_1X_2 + 0.00836\,X_1X_3 + 0.003824\,X_2X_3 \quad (2)$$

3.1.5. Validation of model and optimum conditions

Experimental values show us that these mathematical models can well explain the observed results. According to Joglekar & May (1987), a good mathematical model should predict at least 75 % of the responses; R^2 should then range 0.75 to 1. Our results give the determination coefficient for phenols and flavonoid respectively to be 0.95 and 0.94, falling in the good range, which means our second-order polynomial equations, really represented the experimental data. Also, obtaining values of AADM (analysis of the absolute average deviation) or absolute error of deviation (AED) and Bf (bial factor) respectively equal to 0 and 0.99 for both polyphenolic and flavonoid content, thus confirming the suitability of the models since values were in the normal range (0 for AADM and 0.75<Bf<1.25 for Bf).

Table 4: Evaluation of quadratic model: P value, F value, RC, CF (%), AADM and Bf for phenols and flavonoid

Source	Polyphenolic content				Flavonoid content			
	P value	F value	RC	CF (%)	P value	F value	RC	CF (%)
Solvent ratio(X_1)	**0.005**	12.76	- 0.795	6.18	**0.011**	9.74	- 0.087	5.52
Wavelenght (X_2)	**0.000**	36.45	- 0.121	17.65	**0.000**	28.32	- 0.083	16.04
Time (X_3)	**0.001**	24.96	- 2.693	12.09	**0.000**	53.08	- 1.715	30.06
X_1* X_1	**0.000**	50.79	+ 0.003	22.72	0.346	0.98	+ 0.000	0.11
X_2* X_2	**0.001**	20.27	+ 0.000	10.24	**0.001**	24.64	+ 0.000	13.43
X_3* X_3	0.473	0.56	- 0.010	0.27	0.258	1.44	+ 0.009	0.81
X_1* X_2	0.601	0.29	- 0.000	0.14	0.482	0.53	- 0.000	0.30
X_1* X_3	**0.000**	51.09	+ 0.026	24.74	**0.002**	17.19	+ 0.008	9.74
X_2*X_3	0.158	2.33	+ 0.001	1.13	**0.000**	32.37	+ 0.003	18.33
Validation of the model								
R^2		0.95				0.94		
AADM		0.00				0.00		
Bf		0.99				0.99		

Bold: individual factors that significantly ($p<0.05$) influenced the responses

3.1. Optimization of the process

After validation of the model, the optimal extraction conditions for phenols and flavonnoids were determined using iso-responses and responses surfaces curves. Iso-responses curves are two-dimensional representations showing the variation of a fixed parameter according to two factors. Figures 4,5 and 6 illustrate the variation in polyphenolic content of soybean seeds' extracts under the influence of different factors taken two by two, drawn with Minitab 18.These (Figures)show that maximum content of phenolic compounds is obtained at 120 W, with a solvent ratio of 60:1 for nearly 0 min. the same for flavonoid. More detailed informations are giving by response surfaces curves (figures 7a, b, c and d) on the influence of chosen factors on the studied responses.

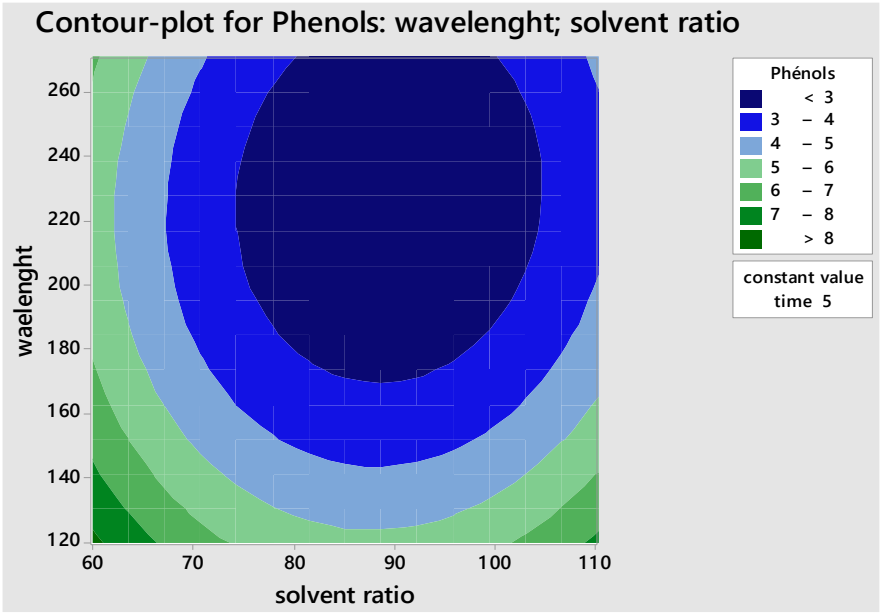


Figure 4: contour-plot for phenols function of avelength and solvent ratio

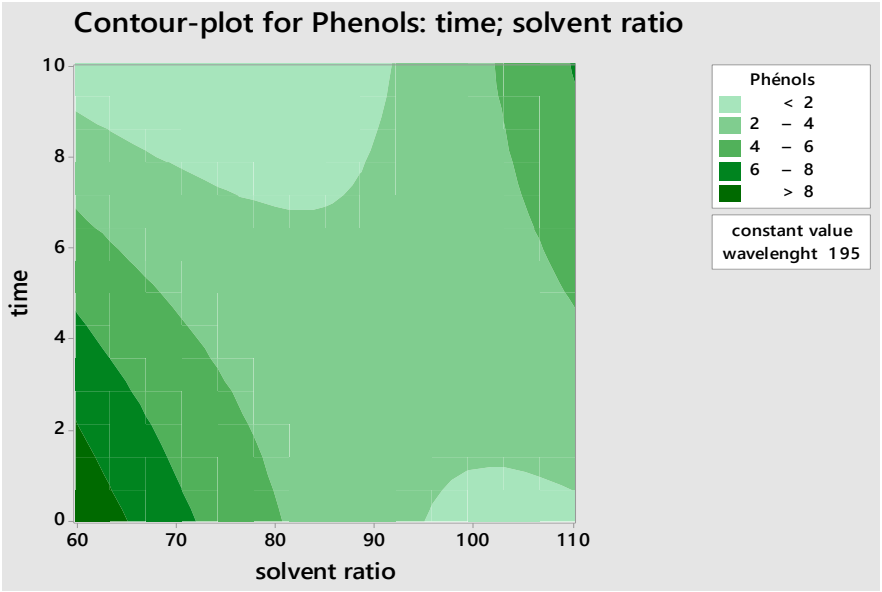


Figure 5: contour-plot for phenols function of time and solvent ratio

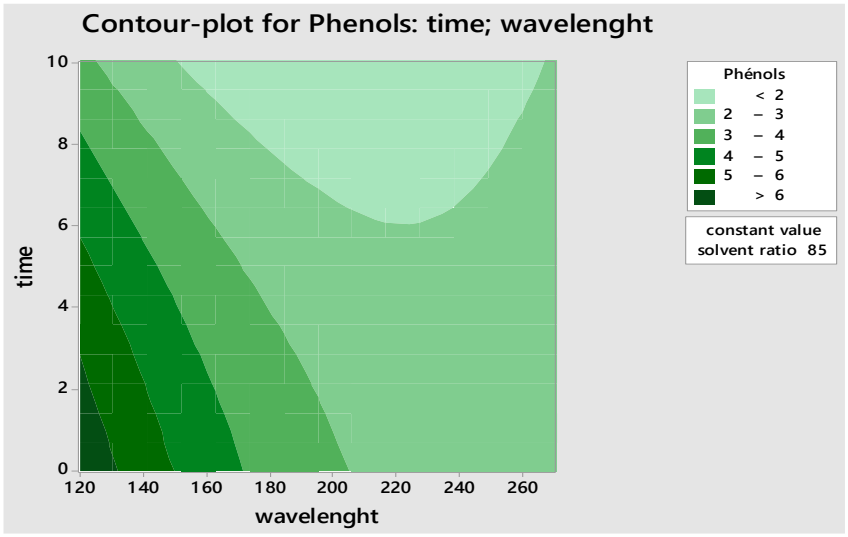
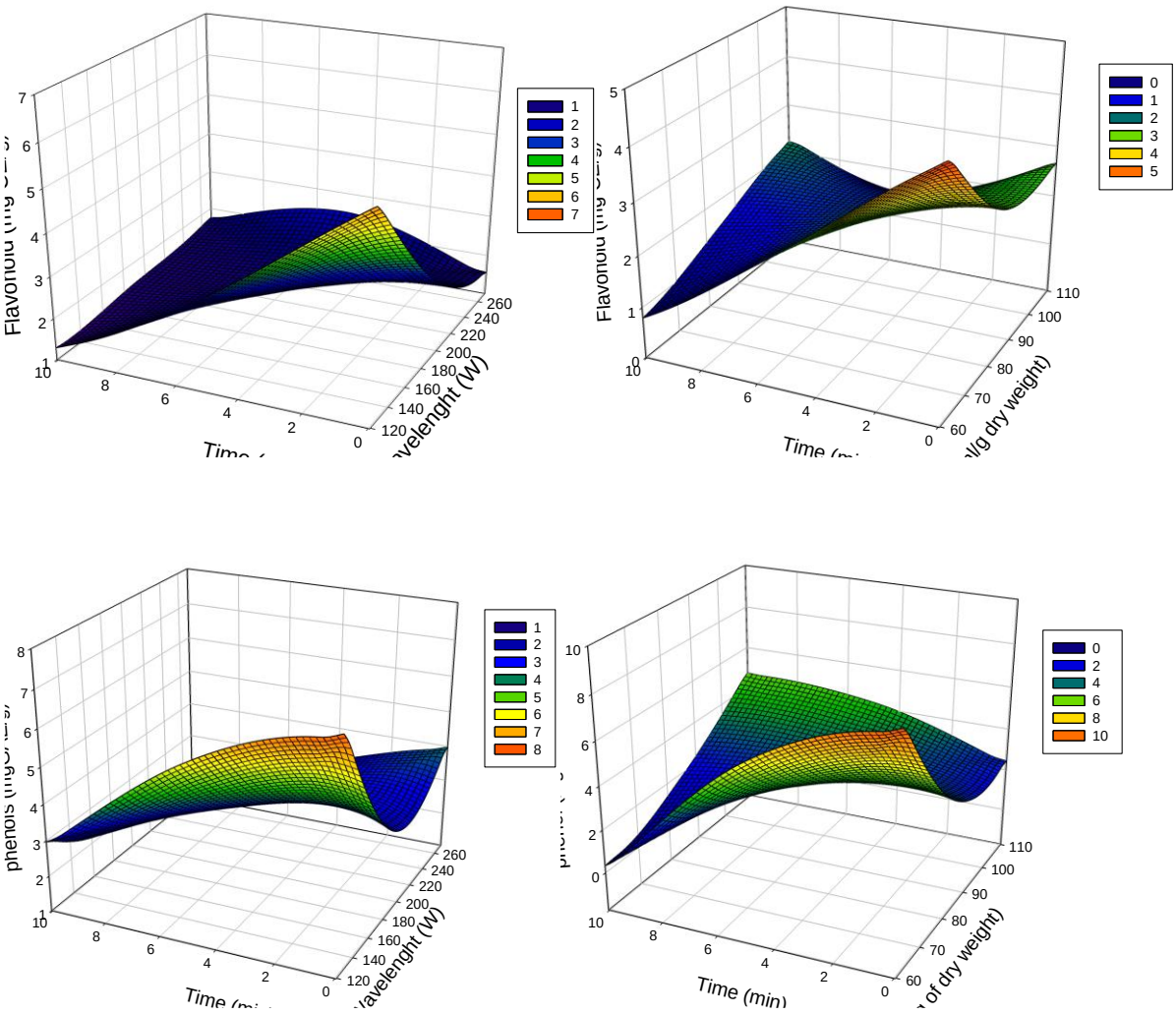


Figure 6: contour-plot for phenols function of time and wavelength



Figures 7 a, b, c and d: responses surfaces curves for flavonoid and phenol content considering the different factors taken 2 by 2

3.2. Confirmation experiments

In order to verify the quality of our model to predict the optimal conditions for our responses, experiments were made, replicating optimal conditions and results compared with the predicted maximal values. So significant differences were noticed between optimal predicted values and experimental values obtained both for polyphenolic and flavonoid contents, thus confirming the validity of the predicted optimal values given by the software.

Table 5: Experimental, predicted values, desirability for phenol and flavonoid in optimal conditions

	Optimal predicted value	Optimal experimental value	Desirability
Phenol (mg GAE/g)	13.09[a]	12.97±0.05 [a]	1.00
Flavonoid (mg CE/g)	7.39 [a]	7.42±0.09 [a]	1.00

On the same line, values with different letters significantly differ ($p>0.05$)

4. CONCLUSION

The study aimed at determining the optimal conditions for extraction of polyphenolic and flavonoid compounds from soybean seeds using a green protocol. RSM was used to determine the conditions and we found out that all factors, namely solvent ratio, time and wavelength significantly influenced both responses. Apart from the solvent ratio which effect tended to reduce the responses when going on any side (higher or lower) of the value 90/1, any increase in other selected factors lead to an almost linear reduction of the responses, suggesting that low solvent, wavelength and time of exposure should be use when attempting to obtain a high polyphenolic and flavonoid content extracts from soybean seeds with MAE. RSM used in this research permitted us to define the conditions for a green extraction of high phenols and flavonnoid from soybean as: 60/1 solvent/dry matter ratio, 120 W wavelength and 0.16 min time.

5. AUTHORS CONTRIBUTION AND FUNDING

Woumbo Cerile Ypolyte and Kuate Dieudonné conceived the work, collected seeds, carried out experimentations, analysed and interpreted data and wrote the paper. Klang Mathilde Julie followed up the work, verified data analysis and read the paper for correction. Womeni Hilaire Macaire supervised the work and read the paper.

All authors have approved the final article.

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6. DECLARATION OF CONFLICT OF INTEREST

None.

7. REFERENCES

Ajay K., Antony J., Navin K., Sharma Rakesh K., 2011. Soybean constituents and their functional benefits, Chapter 12, in Book: *Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry*, ISBN: 978-81-308-0448-4, 367–383.

Ali K., Meredith A., Thomas H., 2013. Techniques for analysis of plant phenolic compounds *Molecules*. ***18***, 2328–2375.

Ana J., Rezica S., Aleksandra S., Vlatka J., Maja M., Antonela M., 2016. Total phenolic and total flavonoid content variability of soybean genotypes in eastern Croatia. *Croatian Journal of Food Science and Technology*, **8**(2) 60–65.

Babu S., Sathiyamoorth S., Palanisamy P., Boopathi S., Rajaram V., 2011. Antioxidant and antimicrobial properties of *Glycine Max*-A review. *I. J. C. B. M. S.*; **1**(2): 49–62.

Djordje M., Milan P., Jegor M., 2007. Phenolic Content and Antioxidant Properties of Soybean (*Glycine max* (L.) Merr.) Seeds. *Molecules*,**12**, 576–581.

Felipe O., 2020. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *J. Food Sci.Technol.*, **57**(12), 4299–4315.

Inglett G., Rose D., Chen D., Stevenson D., Biswas A., 2010. Phenolic content andantioxidant activity of extracts from whole buckwheat (FagopyrumesculentumMöench) with or without microwave irradiation. *Food Chem.*, **119**, 1216–1219.

Joglekar A., May A., 1987. Product excellence through design of experiments. *Cereal Food World*, **32**, 857–868.

Mohamed A., Chang Y., 2008.Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chem.*; 108, 977–985.

Naima R., Hannache H., Oumam M., Sesbou A., Charrier B., Pizzi A., Charrier-El Bouhtoury F., 2019. Green extraction process of tannins obtained from Moroccan Acacia mollissima barks by microwave: Modeling and optimization of the process using the response surface methodology RSM. *Arab. J. Chem.*, **12**, 2668–2684.

Rafiee Z., Jafari S., Alami M., Khomeiri M., 2011. Microwave-assisted extraction of phenolic compounds from olive leaves; a comparison with maceration. *J. Anim. Plant Sci.*, **21**(4), 738–745

Sakthivelu G., Akitha M., Giridhar P., Rajasekaran T., Ravishankar G., Nikolova M., Angelov G., Todorova R., Kosturkova G., *2008.* Isoflavone Composition, Phenolcontent, andantioxidant activity of soybean seeds from India and Bulgaria. *J. Agri. Food Chem.*, **56**, 2090–2095.

Sanja D., Bogdan, N., Nevena L., Jelena J., Andrea S., Nataša Š., Dušan M., Zorica K., 2018. The impact of high-power ultrasound and microwave on the phenolic acid profile and antioxidant activity of the extract from yellow soybean seeds. *Ind. Crops. Prod*, **122**, 223–231.

Shadab G., 2019. Extraction Techniques of Phenolic Compounds from Plants. DOI: 10.5772/intechopen.84705

Vivekananda M., Yogesh M., Hemalatha S., 2007. Microwave Assisted Extraction-An innovative and promising extraction tool for medicinal plant research. *Pharmacogn. Rev.*, **1**(1).

Xuan T., Vo L., Tran Q., Long G., Tran T., Pham T., 2019. Extraction process of polyphenols from soybean (*glycine max* l.) sprouts: optimization and evaluation of antioxidant activity. *Processes*, **7**, 489.