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Optimisation of ultrasound assisted extraction of *T.hyemalis* using the response surface methodology

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Abstract
The present study reports on the extraction of phenolic compounds from aerial parts of *Thymus hyemalis* of Morocco. Box–Behnken Design (BBD), a widely used form of response surface methodology (RSM), was used to investigate the effect of process variables on the ultrasound-assisted extraction (UAE). Three independent variables including ethanol concentration (%), extraction time (min) and solvent-to-material ratio (mL/g) were studied. The results showed that the optimal UAE condition was obtained with an ethanol concentration of 72%, an extraction time of 37 min and a solvent-to-material ratio of 19 mL/g for total phenols, and an ethanol concentration of 70 %, an extraction time of 38 min and solvent-to-material ratio of 20 mL/g for the yield of extraction. The experimental values under optimal conditions were in good consistent with the predicted values.

Keywords: Ultrasound assisted extraction, Optimisation, Phenolic compounds, *Thymus hyemalis*, Response surface methodology

1. Introduction
The genus Thymus consists of approximately 215 species of hardy, perennial and aromatic evergreen or semi-evergreen herbaceous plants distributed throughout Europe, Asia, Africa and Greenland. *Thymus hyemalis* Lange, winter thyme, is an endemic shrub of Morocco, Algeria and Iberian Peninsula [1, 2]. Indeed, in Morocco, *T.hyemalis* was mentioned as endangered and rare species [3].
Few reports on the effect of seasonal variations and environment influences on *T.hyemalis* essential oil composition and biological activities are available [4-7]. In these reports, mainly three different chemotypes of *T.hyemalis* have been revealed namely thymol, thymol/linalool and carvacrol.
Extraction is the initial and the most important step in the recovery and purification of bioactive compounds from plant materials. Many factors such as solvent concentration, extraction temperature, solvent-to-solid ratio, solvent pH, and pressure may significantly influence the extraction efficiency, antioxidant activity and phenolic content [8, 9]. Hence, it is necessary to optimise the extraction conditions to obtain highest yield and phenolic content.
The traditional method of optimisation is laborious and time consuming, since one factor at a time is taken into consideration. In this method, the interactions of various factors are ignored and hence, the chances of obtaining the true optimum conditions are dubious [10, 11]. To overcome this difficulty, usage of statistical optimisation procedure in the form of response surface methodology (RSM) is used [12-17]. RSM enables evaluation of the effects of several factors, as well as the interactions between them [18]. The use of RSM has been successfully used in optimising the extraction of phenolic compounds from food products such as pink guava [19], wheat [10], onion [9], and stink bean [8] among others.
In order to optimise the extraction conditions, including concentration of solvent, extraction time and solvent-to-material ratio, response surface methodology (RSM) has been widely used. By establishing a mathematical model, RSM evaluates multiple parameters and their interactions using quantitative data, effectively optimising complex extraction procedures in a statistical way, thus reducing the number of experimental trials required [20]. Although RSM has been applied to optimise UAE of phenolic compounds in many studies [21-23, 13], to the best of our knowledge, there are no reports yet about the application of RSM on UAE optimisation for the extraction of phenolic compounds from *T.hyemalis*.

In the present work we used RSM to optimise the UAE of phenolic compounds from *T.hyemalis*. The aim of our work was to establish the optimised parameters of UAE for the phenolic compounds extract from *T.hyemalis*, and offer scientific reference for quality assay and utilisation of the resource.

2. Materials and methods

2.1 Plant material and extraction

Plant material of *T.hyemalis* was collected during the flowering stage on the middle Atlas Mountain of Sefrou. The plant materials were identified by experimented botanist (INP 267). Authenticated voucher specimens were deposited in the Herbarium of The National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fez, Morocco. The extraction method was performed with ultrasound cleaning bath Elma – Transsonic TI-H-15. (Frequency of 35 KHz, nominal power 100 W). Extractions were carried out at room temperature. The extracts filtered and concentrated under reduced pressure.

2.2 Experiment design

RSM was used for investigating the influence of three independent variables on total phenols and yield of *T.hyemalis* extracts. The main factors affecting extraction efficiency, including the ethanol concentration (%, X1), extraction time (min, X2) and solvent-to-material ratio (mL/g, X3), were selected as independent variables that should be optimised for the extraction. The temperature was not considered in this present work because the sample was kept at room temperature to avoid the degradation of temperature-sensitive compounds. In the study, the experiments were performed on the Box–Behnken Design (BBD). The coded values of the experimental factors and their levels for the BBD are shown in Table 1.

Table 1. Levels of variables for the experimental design.

Symbols	Independent variables	-1	0	+1
X_1	Ethanol (%)	40	60	80
X_2	Extraction time (min)	20	30	40
X_3	Solvent-to-material ratio (ml/g)	10	20	30

The complete design was carried out in random order and consisted of 17 combinations including five replicates at central point (Table 2). The data from BBD were analysed by multiple regression to fit the following quadratic polynomial model:

$$\mathbf{Y = \beta_0 + \sum \alpha_i X_i + \sum \alpha_{ii} X_i^2 + \sum \alpha_{ij} X_i X_j} \quad \mathbf{(1)}$$

Where Y is the predicted response, β_0 is a constant, $\boldsymbol{\alpha_i}$, $\boldsymbol{\alpha_{ii}}$ and $\boldsymbol{\alpha_{ij}}$ are the linear, quadratic and interactive coefficients of the model, respectively. Accordingly, Xi and Xj represent the levels of the independent variables, respectively.

2.3 Determination of total phenolic content (TPC)

Total phenols were estimated as gallic acid equivalents (GAE), expressed as mg gallic acid/g extract [24]. To ca. 6.0 ml H_2O, 100µl of appropriate concentration of sample were transferred in a 10ml volumetric flask, to which 500µl undiluted Folin-Ciocalteu reagent was subsequently added. After 1min, 1.5ml 20% (w/v) Na_2CO_3 were added and the volume was made up to 10ml with H_2O. After 2h incubation at 25°C, the absorbance was measured at 765nm and compared to gallic acid calibration curve. The data are presented as the average of triplicate analyses.

2.4 Statistical analysis

The experimental results of the response surface design were analysed using Nemrowd software. p Values less than 0.05 were considered to be statistically significant. All experiments were conducted in triplicate unless otherwise noted in the text.

Experimental data for the yields and the contents of phenolics were obtained according to the recommended optimum conditions. The TPC and yields were determined after extraction of phenolic compounds under optimal conditions. The experimental and predicted values were compared in order to determine the validity of the model.

3. Results and Discussion

3.1 Fitting the response surface models

Table 2 shows the yields and TPC of *T.hyemalis* extracts obtained from all experiments. Multiple regression analysis using the quadratic polynomial model (Eq. (1)) was performed based on the results in Table 2. Table 3 presents the results of analysis of variance (ANOVA) and regression coefficients, indicating the contribution of

the quadratic model was significant ($p < 0.05$). The ''fitness'' of the model was investigated through the lack-of-fit test ($p > 0.05$), which indicated the suitability of models to accurately predict the variation [25].

Table 2. Box–Behnken design (uncoded) arrangement for extraction and the responses of yields (%) and TPC (mg GAE/g extract) of *T.hyemalis* extracts

Run	X1 (%)	X2 (min)	X3 (ml/g)	Yield	TPC
1	60	20	30	13	194.06
2	60	30	20	15	201.70
3	60	30	20	15	201.37
4	40	40	20	10	181.76
5	60	30	20	15	201.00
6	60	30	20	14	201.21
7	80	40	20	16	235.69
8	80	30	10	12	192.63
9	40	20	20	11	191.55
10	60	20	10	11	186.78
11	40	30	10	8	150.00
12	60	40	30	13	193.21
13	60	40	10	14	196.40
14	60	30	20	15	201.44
15	40	30	30	6	179.87
16	80	30	30	14	194.21
17	80	20	20	16	222.54

3.2 Effect of extraction parameters on total phenol content

The data shown in Table 3 indicate that the total phenols content and the extraction parameters were quadratic with a good regression coefficient ($R^2 = 0.962$). Fig. 1A-C presents the response surface and contour plots for the influences of extraction parameters on total phenols content. As shown in Fig. 1A, it can be concluded that maximum total phenols extraction could be achieved when the ethanol concentration and extraction time were 72% and 37min, respectively. As the extraction of phenolic compounds depends largely on the polarity of solvents and compounds, a single solvent might not be effective for the extraction of bioactive compound. Hence, a combination of alcohol with water is more effective in extracting phenolic compounds than alcohol alone [26]. The results show that the total phenols content increased with an increase in ethanol concentration from 40% to 72%. This is probably due to the increased solubility of phenolic compounds in the mixture of ethanol and water. The findings obtained from our study are in good agreement with [27], where the phenolic compounds yield from lyophilized fig fruits increased when ethanol concentration increased. It was reported that high phenolic content was obtained when 67% ethanol was used to extract polyphenols from root bark of *Wikstroemiaindica* [11].

The results show also that the total phenols content increased with prolonged extraction time from 20 to 37min. This was understandable because an extended extraction time favours the extraction of phenolic compounds [28].

Fig. 1B presents the interaction of ethanol concentration and solvent-to-material ratio. The increased extraction of polyphenols content was observed with increased solvent-to-material ratio from 10 to 19ml/g. This can be explain by the fact that more solvent can enter cells while more phenolic compounds can permeate into the solvent under the higher solvent-to-material ratio conditions [25].It was reported that liquid/solid ratio (20ml/g) played a significant role in the yield of phenolics, while extraction temperature did not make any significant contribution towards TPC [8].

Fig. 1C presents the interaction of extraction time and the solvent-to-material ratio. It was found that maximum total phenols content was achieved when the extraction time was 37min and the solvent-to-material ratio was 19ml/g.

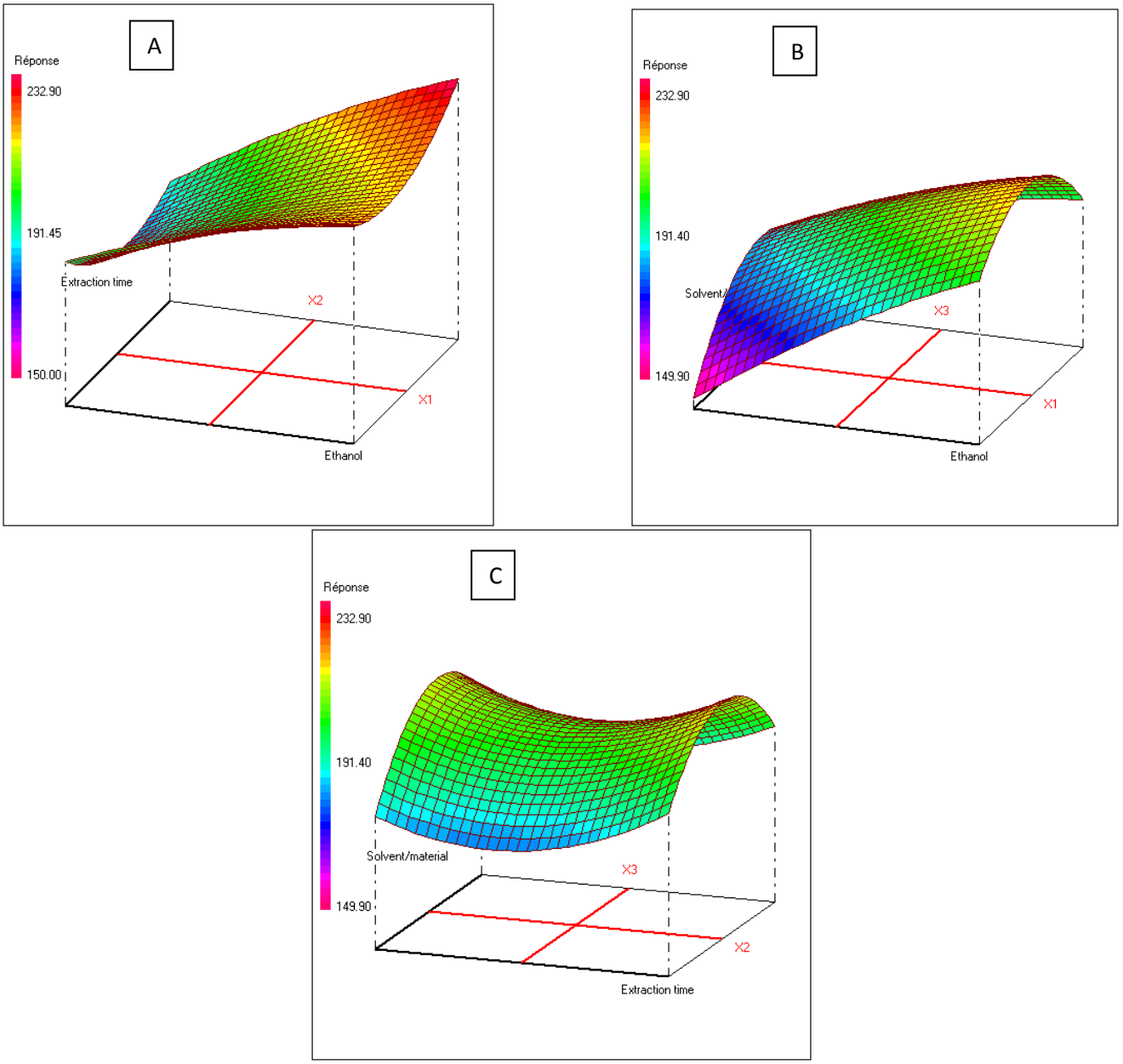

Fig. 1. Response surface plot showing the combined effect of ethanol concentration and extraction time (a), solvent-to-material ratio and ethanol concentration (b) solvent-to-material ratio and extraction time (c) on total phenols content of *T.hyemalis* extract.

3.3 Effect of extraction parameters on the yield

As shown in Table 3, the yields obtained and the extraction parameters were quadratic with a good regression coefficient ($R^2 = 0.976$).

The response surfaces and contour plots shown in Fig.2 demonstrated the changes in the yield obtained as a function of the three variables.

As shown in Fig. 2A, it can be concluded that maximum yield extraction could be achieved when the ethanol concentration and extraction time were 70% and 38min, respectively. The results show that the yield increased with an increase in ethanol concentration from 40% to 70% and with prolonged extraction time from 20 to 38min.

Fig. 2B presents the interaction of ethanol concentration and solvent-to-material ratio. The increased extraction yield was observed with increased solvent-to-material ratio from 10 to 20ml/g.

Fig. 2C presents the interaction of extraction time and the solvent-to-material ratio. It was found that maximum yield was achieved when the extraction time was 37min and the solvent-to-material ratio was 20ml/g.

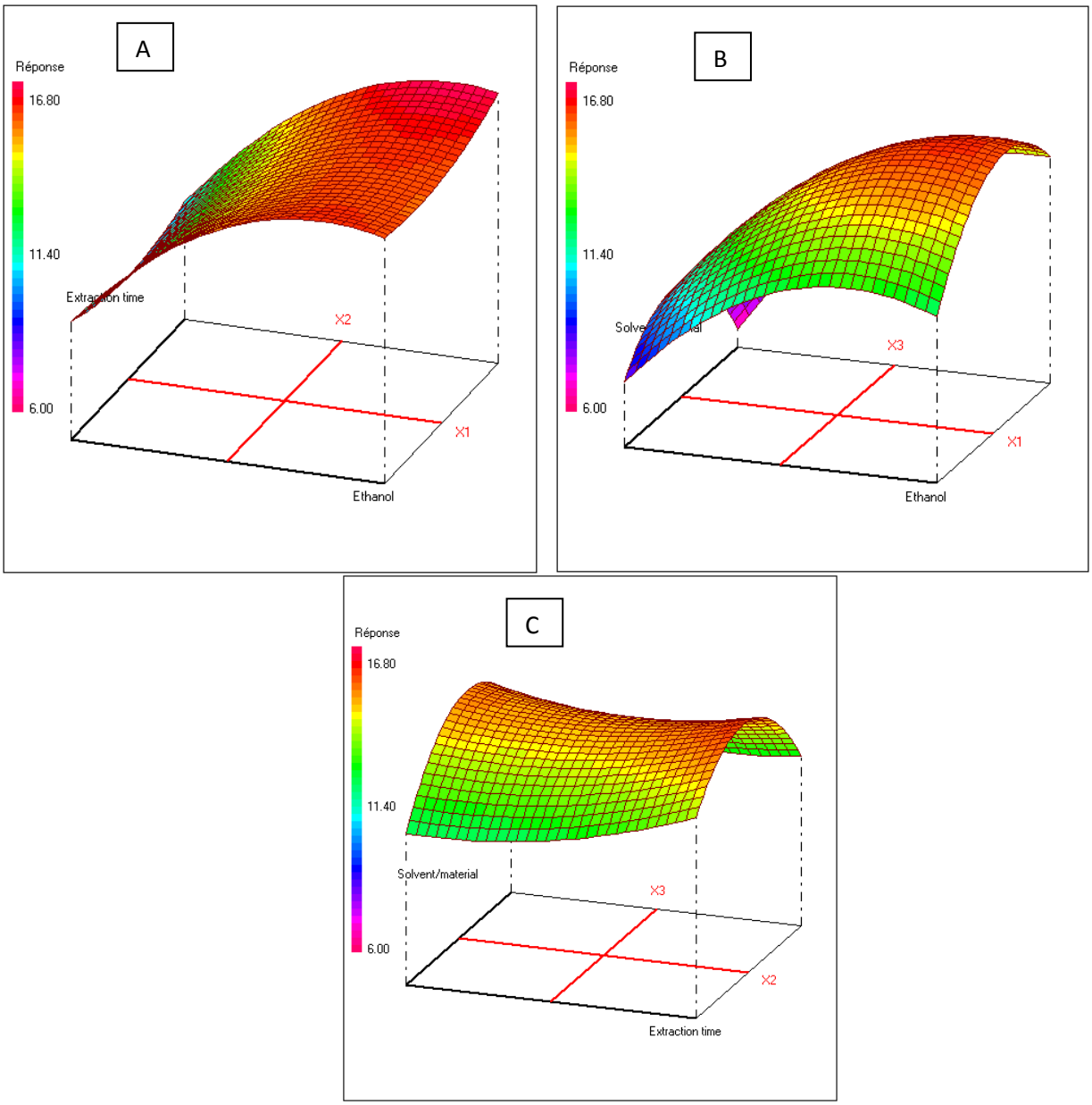

Fig. 2. Response surface plot showing the combined effect of ethanol concentration and extraction time (a), solvent-to-material ratio and ethanol concentration (b) solvent-to-material ratio and extraction time (c) on the yield of *T.hyemalis* extract.

3.4 Verification of predictive model

Based on our experimental results and using the desirability function, an optimisation study was performed to evaluate the optimal ultra-sounds extraction parameters for the yield obtained and the total phenolic content. The target was to obtain *T.hyemalis* extract with high yield and high phenols content.

Three optimal conditions were developed for the two responses, which were ethanol concentration 78%, 40min and 24ml/g. Via the optimum conditions, the corresponding predicted responses of TPC and yield were 225.98 mg GAE/g and 16.35%, respectively. The experiments were run in accordance with the recommended optimum conditions for two responses, to test the adequacy of the surface response model in predicting the optimum response values. The observed values for TPC and yield were 224.97 ± 0.2 mg GAE/g of extract and 16.45 ± 0.36% respectively. The response surface models were verified using experimental and predicted values. No significant difference ($p>0.05$) was found between the experimental and the predicted values for TPC and yield.

Table 3. Results of ANOVA and regression coefficients

Source	TPC	Yield
β_0	201.344	14.800
X_1	17.736	2.875
X_2	1.516	0.250
X_3	4.442	0.125
X_1^2	-3.447	-2.150
X_2^2	9.988	0.600
X_3^2	-18.719	-2.650
X_1X_2	5.735	0.250
X_1X_3	-7.072	1.000
X_2X_3	-2.617	-0.750
R^2	0.962	0.976
R^2adj	0.913	0.946
p-value	< 0.01	0.0176

Conclusion

RSM was successfully employed to optimise the ultrasound extraction conditions of phenolic compounds from *T.hyemalis*. The most efficient extraction conditions were established using a desirability function and were 78%, 40min and 24ml/g of ethanol concentration, extraction time and solvent-to-material ratio respectively.

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References

1. Stahl-Biskup E., Saez F., Medicinal and aromatic plants, *Taylor & Francis*, (2002).
2. Fennane M., Ibn Tattou M., Ouyahya A., El Oualidi J., Flore Pratique du Maroc, *Institut Scientifique*, (2007).
3. Fennane M., Ibn Tattou M., Catalogue des plantes rares, menacées ou endémiques du Maroc, *Palermo Bocconea*, (1998).
4. Jordan M. J., Martinez R. M., Goodner K. L., Baldwin E. A., Sotomayor J. A., *Ind Cop Prod*. 24 (2006) 253.
5. Goodner K. L., Mahattanatawee K., Plotto A., Sotomayor J. A., Jordan M. J., *Ind Crop Prod*. 24 (2006) 264.
6. Saez F., *Biochem. Syst. Ecol*. 23 (1995) 431.
7. Jordan M. J., Martinez R. M., Cases M. A., Sotomayor J. A., *J. Agric. Food Chem*. 5 (2003) 5420.
8. Gan C., Latiff A. A., *Food Chem*. 124 (2011) 1277.
9. Kiassos E., Mylonaki S., Makris D. P., Kefalas P., *IFSET*. 10 (2009) 246.
10. Liyana-Parthirana C., Shahidi F., *Food Chem*. 93 (2005) 47.
11. Lu C., Li Y., Fu Q., Yang L., Jiang J., Zhu J., *Food Chem*. 124 (2011) 1500.
12. Chen F., Sun Y., Zhao G., Liao X., Hu X.,Wu J., Wang Z., *Ultrason. Sonochem*. 14 (2007) 767.
13. Hossain M. B., Brunton N. P., Patras A., Tiwari B., O'Donell C. P., *Ultrason. Sonochem*. 19 (2012) 582.
14. SahinS., Samli R., *Ultrason. Sonochem*. 20 (2013) 595.
15. Zou Y., XieC., Fan G., GuZ., Han Y., *IFSET*. 11 (2010) 611.
16. Zhu C.P., Zhai X.C., Li L.Q., Wu X.X., Li B., *Food Chem*. 177 (2015) 139.
17. Yadini A., El Fakir L., Birich B., El Azzouzi M., El Hajjaji S., *J. Mater. Environ. Sci.* 5 (2014) 2075.
18. Yang B., Zho M., & Jiang Y., *Food Chem*. 110 (2008) 294.
19. Kong K. W., Ismail A. R., Tyug T. S., Prasad K. N., Ismail A., *IFSET*. 45 (2010) 1739.
20. Brachet A., Mateus L., Cherkaoui S., Christen P., Gauvrit J. Y., Lanteri P., Veuthey J. L., *Analusis*. 27 (1999) 772.
21. Skalicka-Woz´ niak K., Szypowski J., Glowniak K., *J. AOAC Int*. 94 (2011) 17.
22. Liu C.W., Wang Y.C., Lu H.C., Chiang W.D., *Process Biochem.* 49 (2014) 1601.
23. Dahmoune F., Nayak B., Moussi K., Remini H., Madani K., *Food Chem*. 166 (2015) 585.
24. Akkol E. K., Goger F., Kosar M., Baser K. H. C., *Food Chem*. 108 (2008) 942.
25. Prasad N. K., Chun Y., En Y., Zhao M., Jiang Y. *IFSET*. 10 (2009) 155.
26. Markom M., Hasan M., Ramli W., Daud W., Singh S., Jahim J. M., *Sep. Pur. Tech*. 52 (2007) 487.
27. Bucić-Kojić A., Planinić M., Tomas S., Jokić S., Mujić I., Bilić M., Velić D., *Pol. J. Food Nutr. Sci*. 61 (2011) 195.
28. Santos B.C., Methods in Polyphenol Analysis, *Williamson*, (2003).