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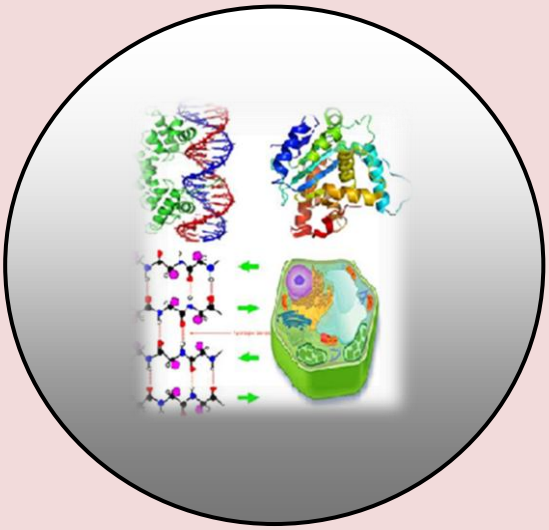

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ABSTRACT

The present work consists to produce a microcapsule powder of total flavonoids and total phenols of methanol extract of the cashew, using a complex coacervation encapsulation method. In the search for optimal conditions for encapsulation, a three level factorial design was set up, while taking into account factors like time and proportions in Arabic gum and gelatin. The kinetic of encapsulation follows a kinetic of 2nd order which gives polynomial equations of the second degree. The conditions found are respectively 45 min, 30% Arabic gum and 70% gelatin, for an encapsulation yield is 84.37%; the encapsulation rate is 77.9% for the total flavonoids and 76.5% for the total phenols. The powder obtained has a doubled concentration in total flavonoids and total phenols than the raw bark powder.

Keywords: Encapsulation, Complex coacervation, Total flavonoids, Total phenols and Anacardium occidentale.

INTRODUCTION

Humans have always used the products of their environment, especially plants for their medical needs. Plants have several therapeutic properties and their uses for the treatment of diseases in living beings are very old and have always been done empirically (Svoboda, and Svoboda, 2000). These plants represent a primary source of medicines and have continued to provide humanity with new remedies to date.

Now, research is showing more and more that the active ingredients in herbal medicines are often linked to secondary metabolites. Thus, the African and Malagasy Council for Higher Education (CAMES) program has empowered the Central Africa Network to carry out research activities on medicinal plants used in the treatment of complicated diseases, with a view to producing improved traditional medicines (Drissa et al., 2010). The performance of extracts in terms of biological activities is linked to extraction techniques. Speaking of extraction methods, apart from the so-called conventional extraction methods, in the context of this work, the innovative method of extraction of flavonoids and polyphenols, natural substances of plant origin has been implemented in order to participate in faster, more efficient technological advances while reducing solvent, energy and sample consumption. This innovative extraction method is microwave assisted extraction (Bétoloum et al., 2018a). This technique can be influenced by parameters such as power, time, polarity of the solvent and the liquid / solid ratio (Asma et al., 2014). So, it is necessary to define the best extraction conditions in terms of biological activities and yield.

To this concern is added the procedure for formulating a powder by complex coacervation of the active extracts of the cashew. Note that the quality and the rate of encapsulation of this powder depend on factors such as the time of mixing and the proportions of adjuvants such as gum Arabic and gelatin. Encapsulation, which is an embedding technique for confining a substance in a polymer matrix covered by one or more semi-permeable membranes, whereby the encapsulated compound becomes more stable than that from which it was isolated. It becomes necessary to resolve the shortcomings noted in the adsorption technique (Goycoolea, 2004). Nowadays, several techniques of microencapsulation of the active ingredients have been developed and described in the literature (Fuchs et al., 2006).

Our objective is to protect the methanolic extract from the bark of the cashew obtained under optimal conditions of microwave assisted extraction against undesirable effects, minimize interactions between the active principle and the polymers of the formulation by coacervation complex (Reineccius, 1995; Tari and Singhal, 2002). To understand this technique, we must study the kinetics and the encapsulation parameters and then look for the most influential levels of factors in complex coacervation.

MATERIAL AND METHODS

Plant Material

Active Ingredient

The active ingredient, called the active Principe, in the formula is the methanol extract from the bark of the plant's trunk. This extract is obtained under optimal conditions of microwave assisted extraction at the time of 83 s, at the power of 620 W, at the solvent-matter ratio of 30 mL / g and at 63% water-methanol for which the values total phenols and total flavonoids, are respectively 655.90 mg EGA / 100 g DM and 82.94 mg EQ / 100 g DM of bark (Betoloum et al., 2018b). After filtration, the filtrate is concentrated using a rotary evaporator and dried in the jars, then crushed, pulverized and stored for further study.

Auxiliaries

The auxiliaries are adjuvants which have secondary and tertiary functions of the formula. These are gum Arabic and gelatin. Gum Arabic is a highly branched hydrocolloid and a polysaccharide polymer.

Its solution has a density of negative charges compared to the acid function (Oumarou et al., 2010). Protein polymer gelatin is used as an encapsulation material thanks to its amphiphilic properties, its ability to interact with different types of molecules, its high molecular weight and the flexibility of its molecular chains. It has a density of positive charges in solution with respect to its amine functions to form ammonium ions (Dickinson and Lopez, 2001).

Preparation of Coacervates

The method used for the preparation of microcapsules is that of Sarunyoo et al., 2018 which has been adapted. The colloidal solution is prepared by mixing the solutions of the two polymers with Moulinex to properly emulsify the solution: the gum Arabic solution (2% m/v) with that of gelatin (8% m/v) at 40°C. The active principle (0.2 g) is introduced into the colloidal solution (1 mL) and the addition of CH_3CO_2H at 50% v/v is made to adjust the pH of the mixture to pH = 4.5 while stirring for 30 min. Then the addition of CH_2O (37% w/v) 4 mL per 100 mL in the mixture allows crosslinking. Finally, the whole is incubated at 4°C for 30 min. Two phases were observed and after screening the coacervates are recovered and subjected to lyophilization to produce the powder of the microcapsules. The freeze-dryer (Scientz-10ND vacuum Freezer driyer) freezes the coacervate solution at -20ºC for 4 hours before producing the powder in 48 hours.

Quantitative Study of Flavonoids and Total Phenols

For quantitative analysis, phytochemical quantification of total flavonoids and total phenols was carried out according to the protocol of Jothi *et al.,* 2013 adapted by Betoloum et al. 2018a.

Study of the Encapsulation Parameters

Effect of time on Encapsulation

Time is a parameter which influences the encapsulation of active extracts by complex coacervation. The mixing was carried out for a period of 0 to 120 min in steps of 15 min, the other parameters being constant. The models studied for modeling are as follows:

The kinetic model of order 2: Its equation is of the form: $c(t) = \frac{t}{k_1 + k_2 * t}$ with c (t) the concentration of the extract at time t, t time d extraction in seconds then k_1 and k_2 the speed constants (Tsatsop et al., 2016). The speed constant k_1 makes it possible to have the speed of the extraction B_0 at the initial instant, that is to say at the instant when the solvent comes into contact with the dry matter: $B_0 = \frac{1}{k_1}$ and the constant of speed k_2 allows to have the quantity of extract at equilibrium: $c_0 = \frac{1}{k_2}$.

Gauss Model (Zheng et al., 2013): It obeys the equation: $Y = ae^{-(\frac{t-t0}{4*k})^2}$ where Y is the rate of encapsulation, a is the maximum value of the encapsulation rate, t is the time studied in the encapsulation, and t_0 is the time corresponding to the maximum value of the encapsulation rate and k is the kinetic constant reflecting the influence of the factor studied on the mixture. Note that this last model was the one best suited for modeling the encapsulation taking into account all of our different factors in the context of this work.

Influence of the proportion of gum Arabic on the encapsulation

The proportion of the gum Arabic solution varies from 0 to 100% in 25% steps and the time remains constant (45 min).

Influence of the proportion of gelatin on the encapsulation

We also varied the proportion of the gelatin solution from 0 to 100% in 25% steps, keeping the time constant (45 min).

Encapsulation Optimization

At the end of the tests, the experimental field for each of the three factors (time (min), proportion of the gum Arabic solution (%) and proportion of the gelatin solution (%)) was chosen. A three-level factorial design is used to find the levels of the most influential factors in complex coacervation (Table 1).

Table 1. Matrix of the factorial plan at three levels (27 experiments).

Factors		Factor levels			Responses		
					FT	PT	Tu
Coded values	X	-1	0	+1			
Real values	X_1						
	X_2						
	X_3						

With X1: gum Arabic; X2: gelatin and X3: mixing time; PT: total phenols; FT: total flavonoids and Tu: turbiddiy.

$X = \frac{X_i - X_0}{\Delta Xi}$ Hence $X_i = X * \Delta X_i + X_0$

This transformation operation use dis that adapted by Jacques Goupy, 2006.

Proposal of a Model

The proposed model has the advantage of properly representing the experimental responses studied in the experimental field of interest and making it possible to obtain an estimate of the value of the studied responses of acceptable quality. The model is as follows:

$Y_i = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_3X_3^2 + \varepsilon$ where Y_i are the expected responses, a_0 the constant, a_1, a_2, a_3 the linear coefficients, a_{11}, a_{22}, a_{33} the square coefficients, a_{12}, a_{13}, a_{23} the interaction coefficients, $X_1, X_2, X_3; X_1X_2, X_1X_3, X_2X_3\ et\ X_1^2, X_2^2, X_3^2$ are the levels of the independent variables, and finally being the error.

Validation of Models

The performance of the model was measured by comparing the values of the predicted responses with those observed. In addition to the linear regression coefficient (R^2), other mathematical procedures and tools were used, the Absolute Analysis of Average Deviation (AADM), the bias factor (Bf) and the Accuracy factors Af_1 (Catherine, 2015) and Af_2 (Baranyi et al., 1999) were determined using the following expressions:

$\mathrm{AADM} = \frac{\sum_{i=1}^{p}\left(\frac{|Y_{iexp} - Y_{ical}|}{Y_{iexp}}\right)}{p}$ with Y_{iexp} the experimental response and Y_{ical} the response calculated from the model for an experiment i; p being the total number of experiments.

$Bf = 10^B$, Bias B is given by the relation: $B = \frac{1}{n}\sum log \left(\frac{Y_{théo}}{Y_{Obs}}\right)$.

$Af_1 = 10^{A_1}$, with A_1 and A_2 the accuracy which is determined according to the following relationships: $A_1 = \frac{1}{n}\sum_{i=1}^{n}\left|\log\left(\frac{Y_{théo}}{Y_{Obs}}\right)\right|$.

Thus, a model is considered perfect if the bias factor and the accuracy factors are equal to the unit, and the AADM equal to zero: $Bf = Af_1 = Af_2 = 1\ et\ AADM = 0$

The graphical representations of the response surfaces of the postulated models were made using the STATGRAPHICS Centurion XV software.

Evaluation of the behaviour of the microcapsule powder

At the end of the encapsulation, a quantitative study of the powder obtained is made. The active ingredient present in microcapsules is characterized by several quantities.

Yield

The most common size of the powders obtained by coacervation is the encapsulation yield which is calculated according to the following formula: $\tau = \frac{m_P}{m_{GA}+m_G+m_{PA}} \times 100$ with m_P the mass of the powder, m_{GA} mass of the gum Arabic, m_G mass of the gelatin and m_{PA} mass of the active ingredient. It is also the yield of the formulation.

Encapsulation Rate

The optimized encapsulation rate of the extract is the content of total phenols or total flavonoids encapsulated on the content of total phenols or total flavonoids introduced expressed as a percentage (%) according to the formula: $\tau = \frac{Q_{encapsulée}}{Q_{introduite}} \times 100$.

RESULTS AND DISCUSSIONS

Study of the influence of factors on coacervation encapsulation

Effect of time on coacervation encapsulation

The influence of time on complex coacervation is shown in Figure 1. The first part of the curves corresponds to an increasing curve from time t = 0 min to time t = 45 min where the optimum is reached at 44.28 NTU of the turbidity of the mixture and the degree of encapsulation of the total phenols and flavonoids respectively at 503.88 mg EGA/100 g P and 62.32 mg EQ/100 g P with a concavity turned upwards at t = 30 min. In this phase, the methanol extract of the bark from the trunk of the cashew is being encapsulated by polymers (gum Arabic and gelatin). The second part corresponds to a decreasing curve at the end of t = 45 min to t = 120 min with an upward concavity at t = 75 min with a reduction in turbidity up to 20.62 NTU and the rate of encapsulation total phenols and flavonoids at 395.82 mg EGA/100 g P and 49.56 mg EQ/100 g P respectively. This phase corresponds to destruction of the microcapsules. The explanation that one could bring to these results is that at t = 45 min, the equilibrium of encapsulation of the active substance of the matrix and the polymers is reached, this is the isoelectric point. Beyond the point, the mixture mixing apparatus breaks the bonds between the polymers and there is degradation of the microcapsules which dissolve in the mixture. The study terminals chosen are [30; 60 min].

Kinetic modeling of encapsulation by coacervation

Two kinetic models allowed us to describe the rate of encapsulation:

- **Gauss model (Zheng et al., 2013)**: $Y = a_0 \exp[-(\frac{t-t_0}{4*k})^2]$ with Y the quantity encapsulated at time t and α_0 the quantity encapsulated at time t_0.
- **Pseudo kinetics of 2nd order (Tsatsop et al., 2016):** $\frac{dq_e}{dt} = k(q_e - qt)^2$ then $q_t = \frac{t}{k_1+k_2\times t}$ with $B_0 = \frac{1}{k_1}$ la initial rate of encapsulation, and $q_e = \frac{1}{k_2}$ the maximum amount encapsulated.

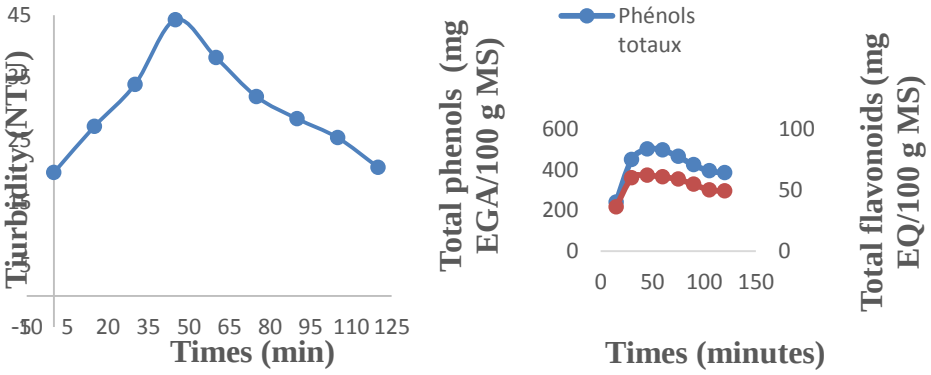


Figure 1. Encapsulation Kinetics.

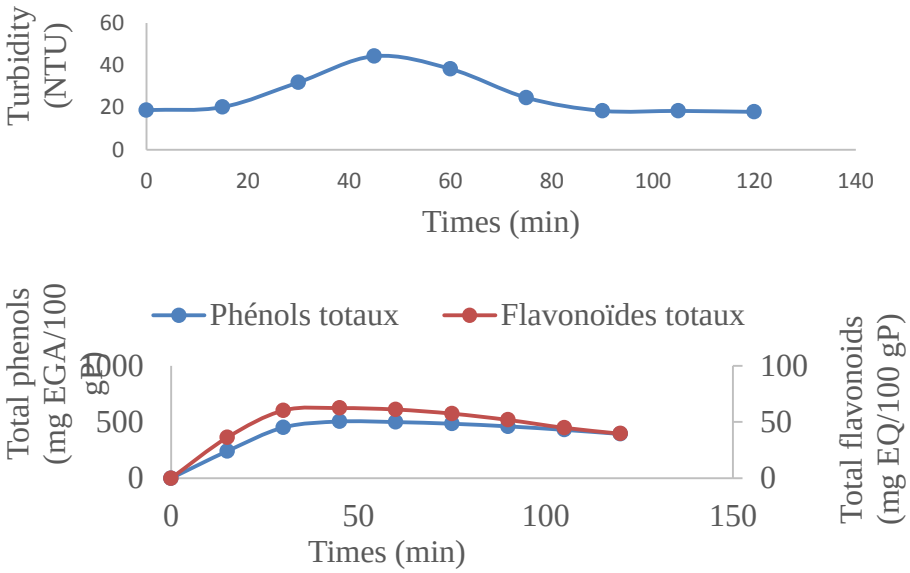


Figure 2. Gauss Model.

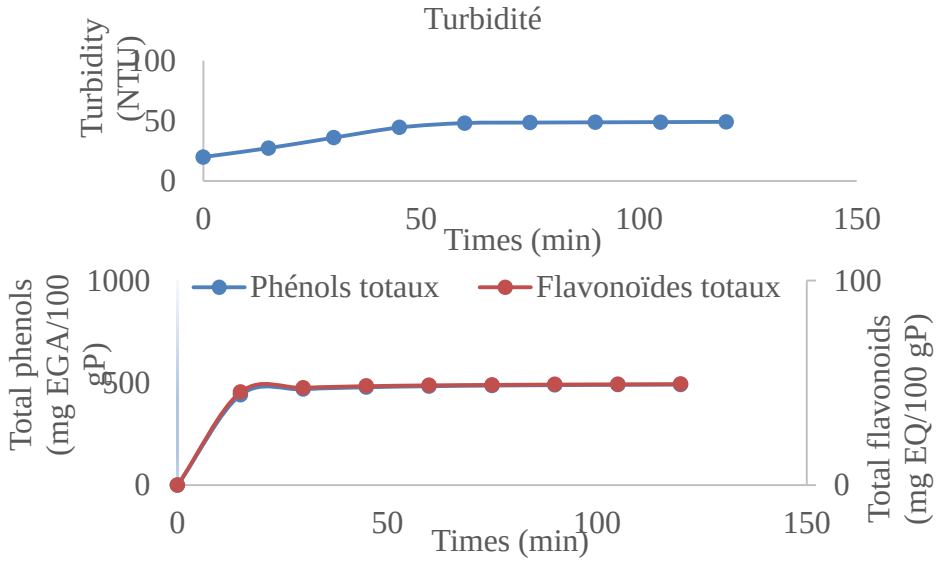


Figure 3. Kinetics Model of Second Order.

Effect of gum Arabic solution on coacervation encapsulation

The figure 4 shows the turbidity of the mixture, total phenols and flavonoids as a function of the proportion of the gum Arabic solution. The mixture is made with a variation of the proportions of the gum Arabic solution and the time, are constants.

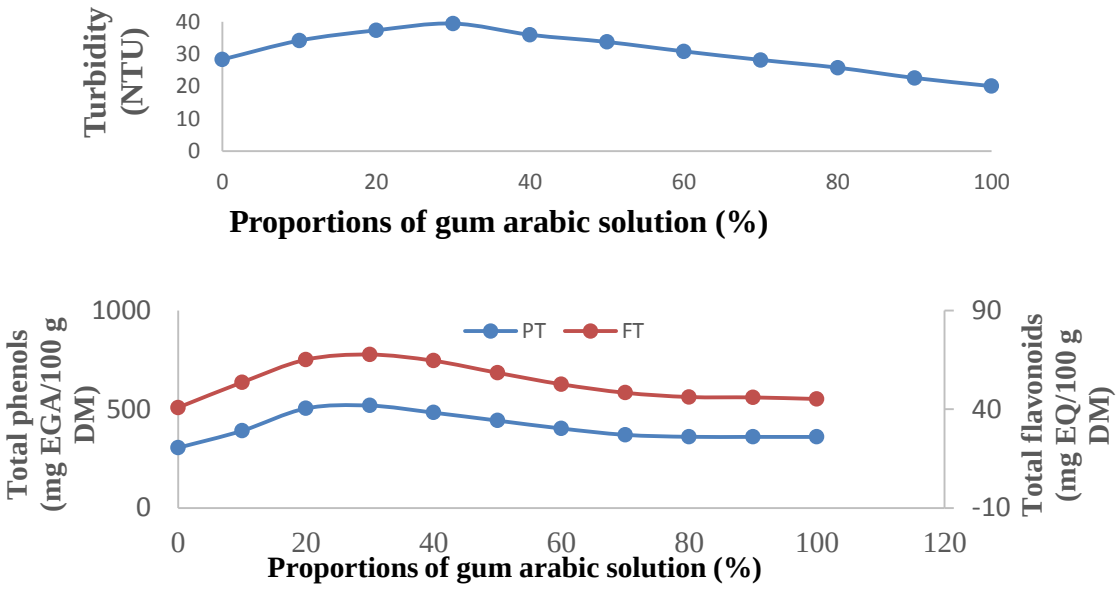
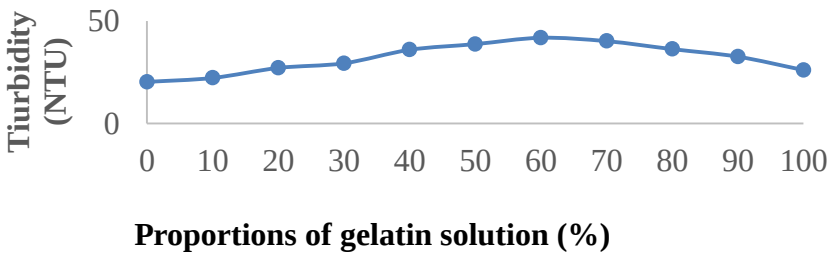


Figure 4. Effect of gum arabic solution on the mixture.

For the effect of gum Arabic, we find that the curves reach their maximum at around 30% which is the equilibrium point then they decrease. The phenomenon of decrease is that the more the gum Arabic is increased, the more the density of negative charges increases and the bonds between the polymers break from where there is degradation of the microcapsules: this is dilution. The limits set for the experiment are [20; 40%].

Effect of gelatin solution on coacervation encapsulation

One of the parameters studied during encapsulation is the effect of the proportion of the gelatin solution. This is done in different proportions and the time is kept constant. Figure 5 below illustrates the evolution of the turbidity of the mixture and that of the encapsulated total phenols and flavonoids.



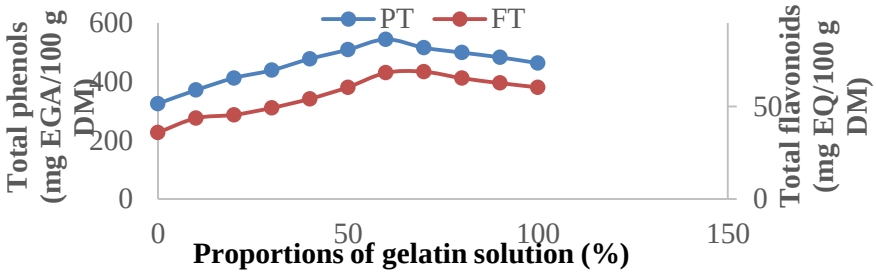


Figure 5. Effect of the gelatin solution on the mixture.

We notice that the pseudo kinetics of 2nd order adjust better because the curves contain an increasing part up to a maximum and an almost constant part for flavonoids and total phenols. As for the Gauss model the curve decreases sharply after their maximum for turbidity. We justify this by the values of the constants in Table 2.

Table 2. Coefficients of kinetic models.

			Pseudo kinetics of 2nd order			Gauss model		
Responses	K_1	K_2	B_0	q_e	R^2	a	k	R^2
Tu	0.05	0.02	19.76	44.38	0.96	44.28	27.7	0.90
FT	0.004	0.002	240.7	503.88	0.97	503.88	106.4	0.93
PT	0.03	0.02	36.22	62.32	0.95	62.32	73.9	0.91

Pseudo kinetics of 2nd order has well described the encapsulation with respect to its R^2 which are superior to those of the Gauss model. However, these two models are valid. So in kinetics of 2nd order, the first phase corresponds to the rapid approximation of molecules (k_1) to coacervation and the second corresponds to the slow approximation (k_2) to crosslinking. The adjustment curves of the Gauss models and kinetics order 2 (Figures 2 and 3) below show that the rate of encapsulation and the turbidity of the mixture depend on the mixing time.

Increasing the amount of gelatin increases the turbidity of the mixture and the content of total phenols and total flavonoids. This could be explained by the gradient of encapsulation of the microcapsules between the polymer and the gelatin matrix which is high when the percentage used is large. The application bounds for the experiment plan adopted are [50; 70%]. Thus, the summary of the preliminary tests is presented in table 3 below.

Table 3. Values of the low and high levels for the four factors chosen.

Factors	Low level	Center	High level
Times (s)	30	45	60
Gum arabic (%)	20	30	40
Gelatine (%)	50	60	70

The specifications which will allow us to define a compromise zone are defined with turbidity greater than or equal to the value of 44.28 NTU, the content of total phenols greater than or equal to 503.88 mg EGA/100 g P and the total flavonoid content greater than or equal to 62.32 mg EQ/100 g P.

Results of the Experiment Plan

At the end of the experiment plan, the statistical analysis allowed us to have the validation indicators of the models designed in Table 4.

Table 4. Validation of models.

Validation Indicator	Model Y_T	Model Y_{PT}	Model Y_{FT}
R^2	99.52	99.63	98.14
Adjusted R^2	99.27	99.43	97.15
AADM	0.01	0.02	0.01
Bias factor	0.894	0.747	0.804
Accuracy factor	1.136	1.003	1.074

For a model to be validated, R^2 must be adjusted ≥ 80%; Bias factor and accuracy factor ϵ [0.75 and 1.25]; and 0 ≤ AADM ≤ 0.3. We have three models which are turbidity model (Y_T), total phenol model (Y_{PT}) and total flavonoid model (Y_{FT}). All three models are valid because their validation indicators are in the standards. The equations of these models are as follows:

Y_T (NTU) = $-74,4 + 0,9X_1 + 0,4X_2 + 4,2X_3 - 0,004X_1X_2 + 0,004X_1X_3 - 0,01X_2X_3 - 0,01X_1^2 + 0,003X_2^2 - 0,04X_3^2$

Y_{PT} (mg EAG/100 gP) = $-763,5 + 11,7X_1 + 2,4X_2 + 46,6X_3 - 0,03X_1X_2 - 0,004X_1X_3 - 0,1X_2X_3 - 0,2X_1^2 + 0,04X_2^2 - 0,46X_3^2$

Y_{FT} (mg EQ/100 gP) = $-166,6 + 0,5X_1 + 2,4X_2 + 6,9X_3 - 0,002X_1X_2 + 0,01X_1X_3 - 0,03X_2X_3 - 0,02X_1^2 - 0,01X_2^2 - 0,1X_3^2$

The factor coefficients are shown in Table 5. All the factors X_1, X_2 and X_3 have a positive and significant influence on the encapsulation. While all interactions and quadratic effects have insignificant influence. Figure 6 presents the contribution of the factors of the models. It confirms that the direct effects contribute to the encapsulation and especially X_3 which contributes strongly, but its quadratic effect contributes negatively. This confirms that the longer the agitation takes place, the more the microcapsules are not broken by the shocks of the agitation.

Table 5. Coefficients of the factors of the models.

Facteurs	$Y_{Turbidity}$	$Y_{Total\ phenols}$	$Y_{Total\ flavonoids}$
X_1	0.9	11.7	0.5
X_2	0.4	2.4	2.4
X_3	4.2	46.6	6.9
X_1X_2	-0.004	-0.03	-0.002
X_1X_3	0.004	-0.004	0.01
X_2X_3	-0.01	-0.1	-0.03
X_1^2	-0.01	-0.2	-0.02
X_2^2	0.003	0.04	-0.01
X_3^2	-0.04	-0.05	-0.1

X_1: Gum arabic; X_2: gelatin and X_3: mixing time

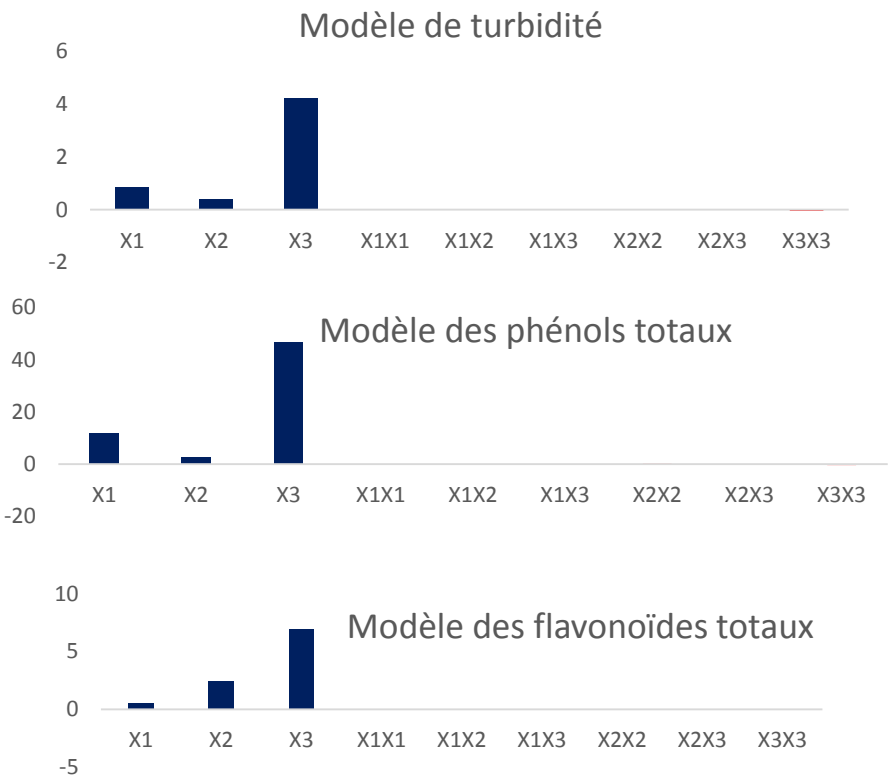


Figure 6. Contribution of Model Factors.

Encapsulation Optimization

The results of the optimization of the encapsulation by complex coacervation are reported in Table 6. The optimum values of responses such as turbidity, total phenols and total flavonoids were measured in two trials and then compared to the values calculated by the equations of the models found. Let's remember that the higher the values, of turbidity, total phenols and total flavonoids, the greater the encapsulation yield and these are the best responses. At each optimal condition, for the responses, experimental tests were carried out and the results obtained are recorded in Table 6. The experimental results are almost similar to the calculated results, which is why the design of these experiments is validated.

Table 6. Optimization results of the encapsulation by complex.

Factor actual values		**$Y_{Turbidity}$ (NTU)**			**Y_{PT} (mg EGA/100 g P)**			**Y_{FT} (mg EQ/100 g P)**		
		Calculated	Test 1	Test 2	Calculated	Test 1	Test 2	Calculated	Test 1	Test 2
X_1 (%)	30	42.61	42.84	43.15	492.94	503.88	506.13	62.32	61.50	64.93
X_2 (%)	70									
X_3 (min)	45									

X_1: proportion of the gum Arabic solution; X_2: proportion of the gelatin solution; X_3: mixing time; $Y_{Turbidity}$: turbidity response; Y_{PT}: response of total phenols and YFT: response of total flavonoids.

In addition, a multi-response optimization was performed. Indeed, the mixture is obtained from the multi-response optimization of the microcapsules. The combination of the different factors is shown in Table 7 below. Under these conditions, we represent the combination of factors for optimizing the encapsulation of microcapsules by coacervation of the models of turbidity, total phenols and total flavonoids in the following table.

Table 7. Combination of factors for multi-response optimization of microcapsule encapsulation models.

Factors	Levels		Turbidity optimum		PT Optimum		FT Optimum	
	Low	High	Coded values	Real Values	Coded values	Real Values	Coded values	Real Values
X_1 (%)	20	40	0.999878	39.999	0.59284	35.9284	-0.99999	20.0001
X_2 (%)	60	80	-0.98351	60.1649	-1.0	60	0.49506	74.9506
X_3 (min)	30	60	1.0	60	1.0	60	1.0	60

X1: proportion of the gum Arabic solution; X2: proportion of the gelatin solution and X3: mixing time.

So for the implementation of our formula, we will take the following conditions: 40% of the gum Arabic solution, 60% of the gelatin solution and the mixing time is 60 min.

Evaluation of the Powder Obtained

Formula yield obtained

The mixture is made according to the experimental plan, by adding in 2 L of distilled water, 40 g of gum arabic, 160 g of gelatin and 40 g of the active Principe (methanol extract from the bark of the trunk of the cashew). After lyophilization, we obtained 202.48 g of powder from the microcapsules. The yield is calculated according to the following formula: $\tau = \frac{m_P}{m_{GA}+m_G+m_{PA}} \times 100$ with m_P the mass of the powder, m_{GA} mass of the gum arabic, m_G mass of the gelatin and m_{PA} mass of the active Principe.

So we have: $\tau = \frac{202,48}{40+160+40} \times 100 = 84,37\%$.

The optimized extract encapsulation rate is the quantity of active compounds encapsulated over the quantity of active compounds introduced, all multiplied by 100, i.e. the content of total phenols and total flavonoids encapsulated on the content of total phenols and total flavonoids introduced expressed as a percentage (%) according to the formula $\tau = \frac{Q_{encapsulée}}{Q_{introduite}} \times 100$. Then $\tau_{FT} = \frac{FT_{encapsulée}}{FT_{introduite}} \times 100 = \frac{49,25}{63,22} \times 100 = 77,90\%$, $\tau_{PT} = \frac{PT_{encapsulée}}{PT_{introduite}} \times 100 = \frac{386,38}{505,01} \times 100 = 76,51\%$.

These values are acceptable because according to (Jessica BILE, 2015) the complex coacervation method encapsulates between 70 to 90%. It is the best chemical method of encapsulation. These results corroborate those of Annalisa et al., 2017.

So we can compare the amount of the active ingredient in the powder obtained and the bark powder of the plant used in our work. Table 8 gives the results of the comparison.

Table 8. Comparison of the quantity of the encapsulated principle with that in the bark.

Extraction technique	Bark powder	Microcapsule powder obtained
Yield (%)	39.71	84.37
FT (mg EQ/100 g P)	262.11	505.01
PT (mg EGA/100 g P)	32.23	63.22

The results show that the powder obtained contains twice as much of the active Principe as the powder of the bark of the trunk of the cashew. So our microcapsule powder is rich and can be valued.

CONCLUSION

We have shown and confirmed the previous studies which attest that the rate of encapsulation of the active compounds depends on the proportion of gum arabic, the proportion of gelatin and also on the mixing time. The kinetics of encapsulation follows kinetics of order 2 which gives polynomial equations of the second degree. The best conditions for encapsulation by complex coacervation are as follows: 40% of the gum arabic solution, 60% of the gelatin solution and the mixing time is 60 min. They give the values of total phenols and total flavonoids of 689.82 mg EAG / 100 g DM and 88.64 mg EQ / 100 g DM, respectively. The evaluation of the powder gives us a yield of the powder formulation of 84.37% and the encapsulation rate of 77.9% for the total flavonoids and 76.5% for the total phenols. Finally, the powder obtained is twice as rich in active principle as the plant material used.

REFERENCES

Annalisa, D., Sara, C. and Gaetano, L. (2017). Encapsulation of Active Molecules in Microparticles Based on Natural Polysaccharides. *First published june 1, 2017. Research Article.* https://doi.org/10.1177/1934578X1701200608.

Baranyi, J., Pin, C. and Ross, T. (1999). Validating and comparing predictive models. *International Journal of Food Microbiology*, 48; 159-166.

Catherine Yardina (2015). Exactitude et intervalles statistiques en validation de méthode. *Laboratoire national de métrologie et d'essais, Trappes, France.* 17th International Congress of Metrology, 02016 (2015). DOI: 10.1051 / metrology / 201502016. © Owned by the authors, published by EDP Sciences, 2015.

Jessica BILE (2015). Microencapsulation d'agent antimicrobien pour le développement de conditionnements primaires fonctionnalisés. https://tel.archives-ouvertes.fr.

Drissa, D., Innocent, P.G., Mahamane, H., Coumbo, T. and Ossy M.J.K. (2010). Recherche sur la Médecine Traditionnelle Africaine : Hypertension. Special issue 14, *African traditional medecine* day, August 31.

Dickinson, E. and G. Lopez (2001). Comparison of the Emulsifying Properties of Fish Gelatin and Commercial Milk Proteins. *Journal of Food Science* 66(1). DOI: 10.1111/j.1365-2621.2001.tb15592.x.

Farid, D., Giorgia, S., Kamal, M., Hocine, R., Asma, C. and Khodir, M. (2014). Pistacia lentiscus leaves as a source of phenolic compounds: Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. *Industrial Crops and Products. Volume 61, November 2014, Pages 31-40.*

Fuchs, M., Turchiuli C. Bohin, M. Cuvelier M. E., Ordonnaud, C., Peyrat-Maillard, M.N. and Dumoulin, E. (2006). *Encapsulation of oil in powder using spray drying and fluidised bed agglomeration* Journal of Food Engineering 75 27–35.

Jacques GOUPY, 2006. © Revue MODULAD - 74 - Numéro 34. Pp.1-43.

López, F., Raúl E. Córdova M.O., F.M. Goycoolea, M.A. Valdez, J.J. Onofre and J.L. Mendoza (2012). Classification and physicochemical characterization of mesquite gum (Prosopis spp.) Yolanda L. *Food Hydrocolloids journal homepage*: *www.elsevier.com/locate/foodhyd*.

Madjitoloum, B.S., Talla, E., Ngassoum, M.B., Tsatsop, T.R.K., Nyemb, J.N. and Mahmout, Y. (2018). Optimization of microwave-assisted extraction of total phenol content and total flavonoids content from *Anacardium occidental L.* (Anacardeaceae) using response surface methodology. *International Journal of Biochemistry and Biotechnology* ISSN 2169-3048 Vol. 7 (4), pp. 800-809.

Madjitoloum, B.S., Talla, E., Nyemb, J.N., Ngassoum, M.B., Tsatsop, T.R.K. and Mahmout, Y. (2018). Comparative survey of three processes used for the extraction of total phenol content and total flavonoid content of *Anacardium occidentale* L. and the assessment of its antioxidant activity. *African Journal of Biotechnology. Vol. 17(40), pp. 1265-1273. DOI: 10.5897/AJB2017.16294. Article Number: EAC480258889. ISSN: 1684-5315. Copyright ©2018. Author(s) retain the copyright of this article. http://www.academicjournals.org/AJB.*

Oumarou, P.M., Régis, P., Oumarou, B., Mama, N. and Nicole, S. (2010). Abandon ou extension des plantations d'acacias au Nord-Cameroun : tout dépendra du fonctionnement des filières gomme arabique. *BOIS ET FORÊT S DES TROPIQUES*, 2010, N°306(4).

Reineccius, G.A. (1995). Liposomes for controlled release in the food industry. In: *Encapsulation and Controlled Release of Food Ingredients* (edited by S.J. Risch&G.A. Reineccius). Pp. 113-131. ACS Symposium Series 590. Washington, DC: American Chemical Society.

Sarunyoo, S., Pichayakarn, Y., Pimrat, P., Duangkhae, M. and Prapaporn, B. (2018). Microencapsulation of citronella oil for mosquito repellent: Preparation and evaluation of release characteristics. Original Article. *Songklanakarin J. Sci. Technol.* 40 (4), 767-775.

Svoboda, K. and Svoboda, T. (2000). Secretory structures of aromatic and medicinal plants. *Microscopix Publications Middle Travelly, Beguildy, Knighton. Powys T07 Luw. V.K.* ISBN 0-953846 1-0-5. Pp.1-30.

Zhang, **S., W. Zhou and H. Qin (2013).** Inverse Gaussian process-based corrosion growth model for energy pipelines considering the sizing error in inspection data. https://doi.org/10.1016/j.corsci.2013.04.020.

Jothi, V., Vijay, K.T., Vasudev, B. and Giliyar, S. (2013). Antimicrobial effect of *Anacardium occidentale* leaf extract against pathogens causing periodontal disease. *Advances in Bioscience and Biotechnology*, 4, 15-18.

Tari, T.A. and R.S. Singhal (2002). Starch-based spherical aggregates: Stability of a model flavouring compound, vanillin entrapped therein. *Carbohydrate Polymers* 50 (4):417-421. DOI: 10.1016/S0144-8617(02)00055-3. *Elsevier.*

Tsatsop, R.K.T., Djiobie, G.T., Kenmogne, B.S., Regonne, K.R. and Ngassoum, M.B. (2016). Optimization of Microwave-Assisted Extraction of Bioactive Compounds from *Anogeissus leiocarpus* Guill. and Perr. Stem Bark Using Response Surface Methodology. *International Journal of Scientific and Technology Research* 5(5):103-112.

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