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Optimization of the Extraction of Gum from *Beilschmieda obscura*

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCE designed the Study, wrote the protocol and wrote the first draft of the manuscript. Authors DMB and SLA managed the literature searches, analyses of the study. Author CMM supervised the study. All authors read and approved the final manuscript

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ABSTRACT

In a view to bring to light Cameroon's rich vegetative potential, optimisation of gum extraction from a local plant *Beilschmiedia obscura* (B.O.) was studied. Extracts of this plant are being used as food improvers in some Cameroonian dishes, but are not yet exploited for industrial applications. Gums were extracted from the seeds of B.O. by solubilisation in aqueous media followed by ethanol purification. A centre composite experimental design with three factors: temperature, pH and alcohol/water ratio was used. Responses considered were, water absorption capacity, solubility index and the emulsifying activity. The results obtained were analysed with the Statistica centurion

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software and the validation criteria for the obtained models where: the AADM[1], the determination coefficient R^2. Sigma plot-11 software was used to plot surface response curves, so as to better visualise the results. From this study, it follows that optimum conditions for the extraction of gums from *Beilschmiedia obscura* (G.B.O.)[2], was attained at pH 7.3, alcohol/water ratio of 3 and at 67.5ºC.

Keywords: Food gums; Beilschmiedia obscura; extraction; optimisation.

1. INTRODUCTION

Generally, plants serve as a source of raw material for the manufacture of water-soluble gums. These substances are used in cosmetics, therapeutics, textile industry and the food industry. The main industrial sources of water-soluble gums of vegetative origin are: the brown algae (agar, alginates) originating from England, the red algae (carraghenanes) originating from Ireland, the carob seeds of Egyptian origin, the guar seeds of Indian origin (galactomannanes), foreign fruits and vegetables more precisely apples (pectins) [1]. The works of Ndjouenkeu [2] has demonstrated the presence of gums in the seeds of *Beilschmiedia sp,* plant of Central and Western Africa. But they are only used for human consumption [3,4,5], despite the high vegetative diversity and industrial potential, their industrial exploitation is still rudimentary in Cameroon. In order to promote the industrial application of this local plant, this study had as main objective to optimise the extraction process and the characterisation of gums obtained from *Beilschmiedia obscura*.

Otherwise, Water-soluble gums have the advantage of being soluble in water. Being polysaccharides, those of vegetative origin are generally affected by temperature and pH. Indeed the temperature and the pH of gum extraction vary according to their chemical composition. Non ionised gums resulting from seeds are more soluble at a pH range of 6 to 9. As for their extraction temperature, it varies between 50 and 85ºC, in order to limit degradations due to severe heat treatment [6]. For pectins, the extraction pH is rather acidic because of their great stability in acid medium due to their high percentage of galacturonic acid. Their extraction temperature generally lies between 60 and 100ºC [7]. The extraction yield is thus strongly affected by the pH and the extraction temperature.

Moreover, Alcohol/water ratio is a factor to consider for the extraction of gums. Indeed being an organic solvent, alcohol facilitates the precipitation of polysaccharides which are insoluble in organic solvents [8,9]. However, the quantity of gums precipitated depends strongly on the alcohol/water ratio and the interactions between the various components of the raw material during the purification of gums, hence the importance of this factor in gum extraction. Generally gum extraction depends on the temperature, pH and alcohol/water ratio for which gums extracted have different yields and properties; it is the reason to study how these factors affect the extraction of water soluble gums form *Beilschmiedia obscura* seeds.

2. MATERIALS AND METHODS

2.1 Sampling of the *Beilschmiedia obscura* Seeds

Dried seeds of *Beilschmiedia obscura* from Meiganga, a town located 160 km from Ngaoundere (Adamaoua, Cameroon) were purchased from a local market in the

[1] *Absolute Mean Deviation Analyses*
[2] *Gum of Beilschmiedia obscura*

Ngaoundere Town. They were packed in polyethylene bags and transported to the Physicochemistry laboratory of ENSAI, University of Ngaoundere. These seeds were cleaned and washed with distilled water, then dried for 48 hours at 40ºC with an electric dryer (P. Dominioni Lurate caccivio como, Italy). After drying, the seeds were crushed with a cereal crusher. The powder obtained was then sieved with a 400 µm sieve and the fine flour was preserved in airtight containers prior to gum extraction.

2.2 Extraction Method

The method used for extraction was adopted from the works of Ndjouenkeu [2] and Vinod [6], within the framework of this study, ethanol precipitation at 95º was done. The wet gums obtained were then dried with an electric dryer. After drying, the gums were preserved in polyethylene bags.

2.3 Stages of Gum Extraction

In a more detailed manner, the various stages for the extraction process of gums of B.O can be described as follow:

2.4 Preparation of the Extraction Solution

10g of sample was weighed out on an analytical balance (Denver Instrument, model Apx-3202) and each dispensed into 250 ml of distilled water, to obtain a solution with a final concentration of 10g/250ml. In order to optimise the extraction of gums, a centre composite experimental design was used to allow the variation of pH within experimental limits. A pH range between 6 and 9 was used. The experiments carried out were represented on an experimental matrix as shown on Table 1. The solutions were buffered with 2M citric acid solutions and 0.5 M sodium hydrogen carbonate solution with the help of a pH-meter within a temperature range of 50 to 85ºC for B.O. gums.

Table 1. *Beilschmiedia obscura* gum extraction matrix

Trials	Temperature (ºC)	pH
1	50.0	6.0
2	85.0	6.0
3	50.0	9.0
4	85.0	9.0
5	50.0	6.0
6	85.0	6.0
7	50.0	9.0
8	85.0	9.0
9	67.5	7.5
10	67.5	7.5
11	67.5	7.5
12	38.0	7.5
13	97.0	7.5
14	67.5	5.0
15	67.5	10.0
16	67.5	7.5
17	67.5	7.5

2.4.1 Extraction

Extraction was done based on the conditions described by the experimental matrix presented on Table 1. A centred composite plan was used for the optimisation of the extraction. Solutions at different temperatures were placed under agitation at a speed of 3600 rpm for 30 minutes; the optimisation matrices for the extraction were as follows:

2.4.2 Centrifugation

After extraction, the solutions were centrifuged at 3600 rpm for 15 minutes; the sediments were subjected to a second extraction under the same conditions as previously described, followed by a second centrifugation (3600 rpm for 15 min) stage. The resulting supernatants from the two successive extractions were considered to be the solubilised gums.

2.4.3 Purification

Purification was done with 95º ethanol. After measuring the volume of the supernatant, precipitation was carried out by incorporating a given volume of ethanol into the medium. The alcohol/water ratio was range from 1 to 3 and the precipitation time was fixed at 30 minutes. This operation was optimised by the use of a centred composite experimental plan (Table 2). After the precipitation, the gums were then separated by filtration. They were washed with ethanol in order to eliminate the impurities.

Table 2. Purification matrix of *Beilschmiedia obscura* gums

Trials	Alcohol/water ratio
1	1.0
2	1.0
3	1.0
4	1.0
5	3.0
6	3.0
7	3.0
8	3.0
9	2.0
10	2.0
11	2.0
12	2.0
13	2.0
14	2.0
15	2.0
16	0.3
17	3.7

2.4.4 Drying

The gums were dried at 38ºC for 15 hours with an electric dryer. The gums were then crushed in order to obtain a powder which was conditioned in airtight packages. The experiment was repeated thrice. Fig. 1 illustrates the extraction method.

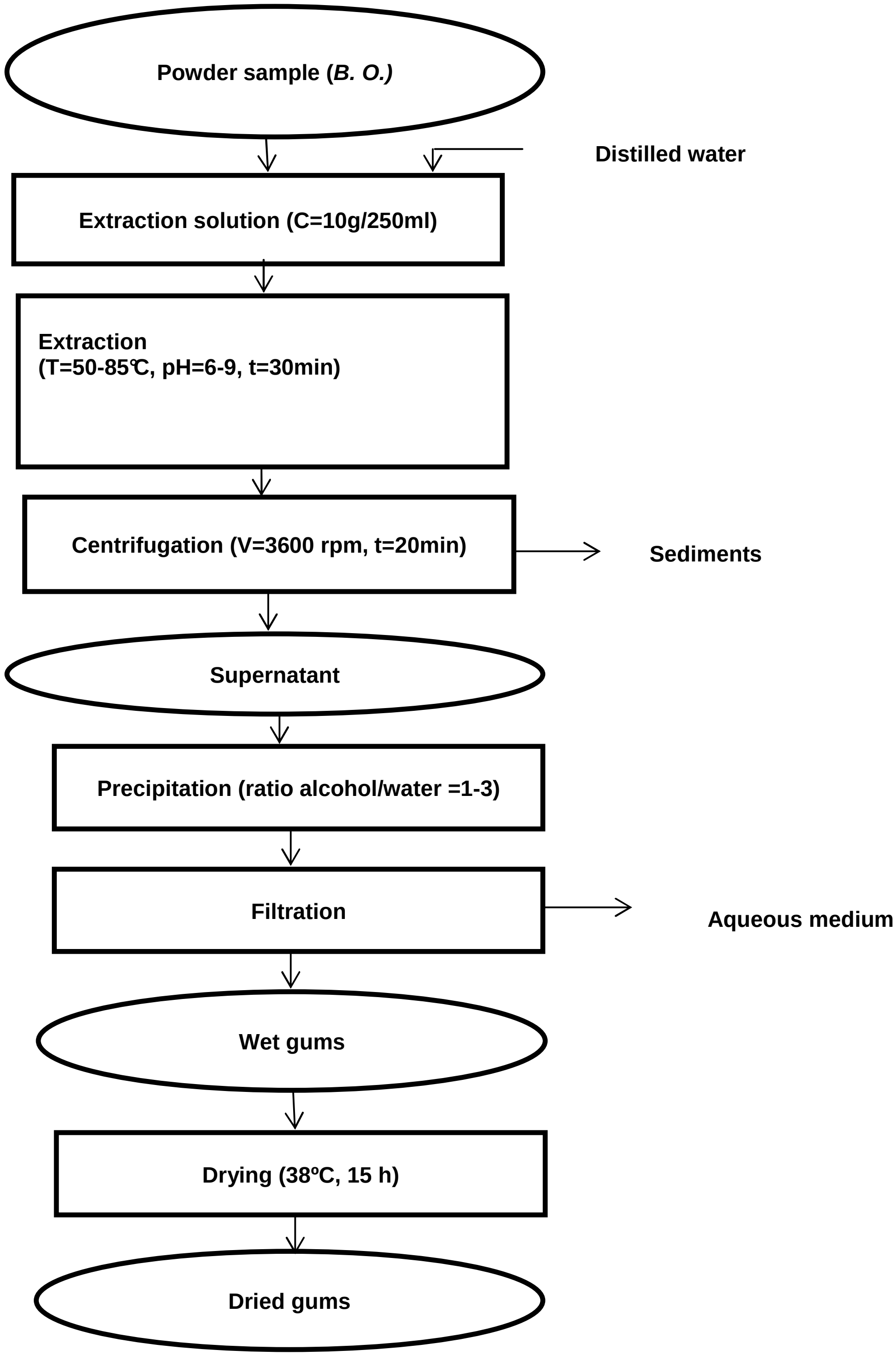

Fig. 1. Extraction process for the gums of *Beilschmiedia obscura*

2.5 Determination of the Extraction Yield

The extraction yield (Y) determines the proportion or the percentage of gum obtained from the raw material for each experiment. It was thus calculated:

$$Y(\%) = \frac{(m1 - m2)}{m1} x100\%$$

Where:

- m_1: mass of sample; - m_2: mass of gum

2.6 Determination of the Absorbency Apparent Water (aaw)

The water absorption capacity is the ability of a substance to adsorb unto water molecules. The method used was adapted from that of Philips et al. [10]. 0.4g of corn starch powder was mixed with 0.1 g of gum powder. This mixture of powders (m1) with a total mass of 0.5g was mixed with 10 ml distilled water, agitated for 30 minutes and centrifuged at 5600 turns/min for 30 minutes in a centrifuge (standard DL -6000). The recovered sediment (m2) was weighed and the absorbency apparent water (aaw)[3] was thus calculated:

$$aaw(gwater / gSample) = \frac{(m2 - m1)}{m1} x100$$

2.7 Determination of the Solubility Index

The solubility index (SI) expresses the percentage of dissolved gum in a given volume of water compared to the mass of dry raw material. It was done according to the method of Anderson et al. [11]. 0.1g of gum was added to 0.4 g of corn starch and the powder mixture was dissolved into 10 ml of water and agitated for 30mins. The homogenate was centrifuged at 5600turns /min for 30mins.The sediments m2 was recovered, weighed and dried at 105℃ in an oven for 24h. The weight of the dry sediments (m3) was determined and the solubility index was thus determined:

SI (%) = MSe-[((m3 - Mo) X 100)/(m2 - Mo]

$$IS(\%) = Mse - \frac{m3 - mo}{m2 - mo} x100$$

MSe is the dry matter of the sample

2.8 Determination of the Gum Emulsifying Properties

The emulsifying properties of a gum determine its capacity to allow the miscibility of two dissimilar liquids. In order to verify the presence of hydrophilic and hydrophobic groups, emulsions were prepared as described above and emulsifying activity was determined.

[3] *absorbency apparent water (aaw)*

2.8.1 Preparation of the emulsions

0.1 g of gum was dissolved in 5ml of water, and then mixed with 5ml of cotton seed oil under agitation for 30 mins with a magnetic stirrer. The mixture thus obtained constituted the emulsion used to determine the emulsification activity.

2.8.2 Estimation of the emulsifying activity

The emulsifying activity was evaluated by the method adapted from Muschiolik [12]. 10 ml of the emulsion was introduced into a graduated tube and left at rest for 30 minutes. The volume (height) of the emulsified phase was measured and the gum emulsification activity (EA)[4] in percentage was thus determined:

$$EA(\%) = \frac{He}{Hw} x100\,\%$$

- *He* is the height of the emulsified layer
- *Hw* is the total height of the liquid in the tube.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

The yields were determined by varying the factor temperature, pH and alcohol/water ratio. The results of the optimisation of the extraction of B.O. gum are presented in Table 3; this Table also gives the values of the predicted yields and the residuals.

The extraction yields are a function of the extraction factor. It was observed that the centre experiments had yields of 4.91, 4.20 and 4.75 % corresponding to a standard deviation of 0.37. Experimental sets : 1, 4, 6, 7, 8, 9 and 11, 14, 15 16 had observed yields higher than the predicted yields while experiments 2, 3, 5, 10, 12, 13 and 17 had observed yields lower than the predicted yields. This could be due to experimental errors and the use of ethanol stocks of different qualities. The sum of residuals amounted to 0.0000006, a value appreciably equal to 0. This result confirms the reliability of the model. Experiment 15 had the highest yield; this corresponds to experimental conditions of temperature 85ºC, pH 6 and alcohol/water ratio of 3. Experiment 16, which corresponded to experimental conditions of temperature 67.5ºC, pH 7.5 and a ratio of 0.3, had no extraction. It is important to note that the yield found during this work was lower than that found by Ndjouenkeu [2]. Indeed the latter obtained a 53% gum yield from *Beilschmiedia sp*. It should be noted that in his study, the percentage of gums is determined by the total percentage of the polysaccharide starting material. For of this work, the percentage of gums is determined by the ratio between the mass of the raw material and the mass of dry gum obtained.

This result is also different from those of Saidou [4] he has isolated gums from *Triumfetta corifiolia* and *Bridelia thermifolia*. This can be explained by the fact that this author did successive infusion of the same sample during the extraction.

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[4]*emulsification activity*

Table 3. Presentation of the extraction yields of the gum of B.O

Trials	T (ºC)	pH	Alcohol/water ratio	Observed yields	Predicted yields	Residuals
1	50.0	6	1.0	1.9	1.570	0.329
2	85.0	6	1.0	0.8	2.366	-1.566
3	50.0	9	1.0	2.1	2.652	-0.552
4	85.0	9	1.0	0.5	-0.436	0.9367
5	50.0	6	3.0	4.9	5.314	-0.414
6	85.0	6	3.0	11.4	10.323	1.074
7	50.0	9	3.0	6.6	4.511	2.088
8	85.0	9	3.0	5.83	5.637	0.192
9	67.5	7.5	2.0	4.91	4.577	0.332
10	67.5	7.5	2.0	4.2	4.577	-0.377
11	67.5	7.5	2.0	4.75	4.577	0.172
12	38.0	7.5	2.0	3.2	3.811	-0.611
13	97.0	7.5	2.0	5.3	5.427	-0.127
14	67.5	5.0	2.0	1.0747	5.605	0.594
15	67.5	10.0	2.0	1.24	2.573	-1.333
16	67.5	7.5	0.3	0.0	-0.758	0.758
17	67.5	7.5	3.7	6.0	7.497	-1.497

3.2 Equation of the Model

$$Y = 4.57775 + 0.960948X1 - 1.803X2 + 4.909X3 + 0.029425X1^2 - 1.9425X1\,X2 + 2.1075X1\,X3 - 0.345341X2^2 - 0.9425\,X2\,X3 - 0.854455\,X3^2$$

X1 = Temperature; X2 = pH; X3 = Alcohol/water ratio

This equation had a constant of 4.57775, which corresponded to the average yields obtained. It was revealed that the alcohol/water ratio factor affected the extraction yield to a greater extent, because it had the largest positive experimental coefficient 4.909. Hence, the yield increased proportionately with the quantity of alcohol. The factor alcohol/water ratio was followed by the factor pH which had a coefficient of -1.803. Its effect is negative on the yield; thus an increase in the pH within the experimental domain led to a decrease in the yield. The factor temperature had a positive effect but its effect was lower than those of the other factors as could be seen from the value of the coefficients. The interaction temperature - ratio had the greatest effect on the yield with a coefficient of 2.1075. Its effect was positive followed by the interaction temperature - pH which had a coefficient of -1.9425; this indicates a significant and negative impact on the extraction yield. The quadratic interaction ratio-ratio followed; with a negative effect has a coefficient of -0.845544. The interaction pH-ratio had a lower impact (negative) on the extraction yield with a coefficient of -0.9425. It is thus clear that at lower pH values and high temperatures combined with higher alcohol/water ratios respectively improved extraction of B.O. gums. The alcohol/water ratio had a highly significant effect; followed by the factor pH. The factor temperature and the interactions do not have a significant effect on the extraction yield. This implies that, only the variations of the ratio and the pH had a dominating impact on the extraction yield.

3.3 Validation of the Model

The R^2 and the AADM were calculated and used as validation criteria for the model (Table 4)

Table 4. Validation criteria for the gum of B.O. yield

Validation elements	Abbreviation	Real values	Standard values
Determination coefficient	R^2	88.585%	100%
Absolute mean deviation analyses	AADM	0.38974881	0

Despite the facts that R2 was 88.585, the computed and standard value for the AADM was similar and allows the validation of this model. Surface response graphs were plotted with the sigma plot-11 software. The yields obtained were a function of the alcohol/water ratio and the pH at a fixed temperature within the experimental domain. Figs. 2-3 represent the surface response curves for the yield as a function of the pH and the ratio.

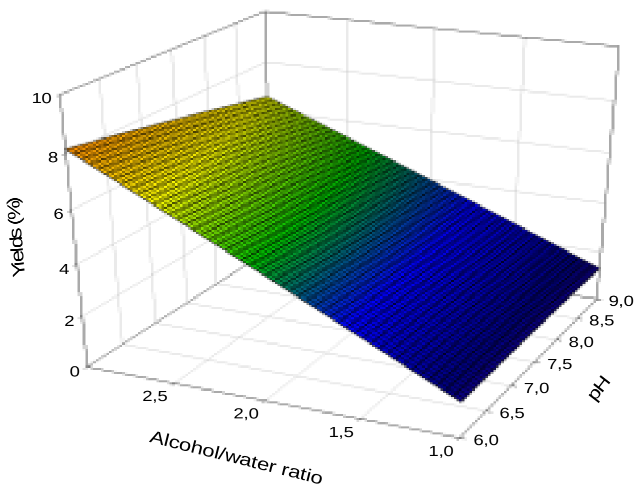

Fig. 2. Evaluation of the gum of B.O. yield as a function of the alcohol/water ratio and pH

■ : 0 %; ■ : 2% ; ■ : 4% ; ■ : 6% ; ■ : 8% ; ■ : 10% ; ■ : 12% ; ■ : 14%

From these surface curves it follows that the gums of *B.O*. were optimally extracted at pH between 5 and 6, alcohol/water ratio between 3 and 3.5 and temperature between 80 and 100ºC. Indeed, the yield increases with an increase in the alcohol/water ratio, an increase in the temperature and a decrease in the pH. In fact, high temperatures favour the breakdown of bonds within polysaccharides and other macromolecules, thus favouring their solubilisation in water by exposing hydrophilic groups which are capable of bonding with water. At pH between 5 and 6 the gum of B.O adopts a favourable conformation which facilitates its release in the medium. In addition, the greater the volume of ethanol, the more the precipitation of gums. Indeed the polysaccharide gums are insoluble in organic solvents such as ethanol; so one will expect that the greater the quantities of alcohol present, the more the precipitation of gums are favoured.

3.4 Absorbency Apparent Water (aaw)

aaw was determined under given conditions of extraction of temperature, pH and the alcohol/water ratio. Table 5 presents the results of observed aaw, those predicted by the model and the residuals.

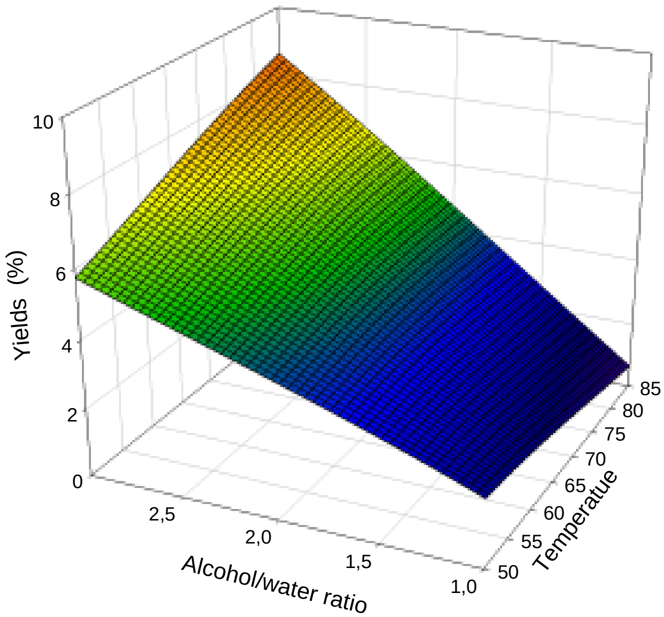

Fig. 3. Evaluation of the gum of B.O. yield as a function of the ratio and temperature
■ : 0% ; ■ : 2% ; ■ : 4% ; ■ : 6% ; ■ : 8% ; ■ : 10% ; ■ : 12% ; ■ : 14%

It follows from this table that the experiments at the centre of the experimental domain correspond to aaw values of 332, 328 and 330 %; with a standard deviation of 1.41. Experiments 1, 2, 5, 6, 8, 9, 11, 12, 14, 16 and 17 have observed aaw values higher than the predicted aaw; while experiments 3, 4, 7, 10, 13, and 15 have observed aaw values lower than the predicted aaw. These could be due to experimental errors and variations in the quality of reagents used. In addition, the sum of the residuals was equal to -0.002, a value appreciably equal to 0. Hence, these results can thus be validated. The lowest aaw value was obtained with experiment 16 and corresponds to a temperature of 67.5ºC, pH of 7.5 and a ratio of 0.3. Optimal aaw was obtained with experiment 6 at a temperature of 50ºC, pH of 9 and an alcohol/water ratio of 1. Indeed these conditions impart a favourable configuration to the B.O. gums, hence the possibility of easily binding to water. The aaw of the blank sample was 68g water per gram of gum. This value was very small compared to those obtained for the gums of B.O. whose optimum value was 700g water/g of gum. These gums thus have a significant aaw and can consequently be used in bread-making to increase the aaw of the flours and thus improve the consistency of these flours; hence the higher the aaw the better the yield.

3.5 Equation of the Model

$$Y = 316.687 - 171.102X1 + 81.171X2 + 62.824X3 + 159.137X1^2 - 13.5X1\,X2 + 2.5X1\,X3 - 89.133\,X2^2 - 88.5X2\,X3 - 38.1455X_3{}^2$$

The constant of the aaw model obtained was 316.687 which represent the average of the aaw obtained. aaw was negatively influenced by temperature, affecting the response to a greater extent with a coefficient of -171.102. It was closely followed by pH which had a positive effect and a coefficient of 81.171.The ratio positively affects aaw, and had a coefficient of 62.824 thus having the least effect on aaw, while the quadratic interaction temperature-temperature had the greatest effect on aaw. It had a positive effect and a coefficient of 159.13, followed by the interaction pH-ratio with a negative effect on aaw and a coefficient of -88.5. The quadratic interaction ratio followed closely with a negative effect, thus, a coefficient of -38.1455. The interaction temperature-ratio (negative effect) and temperature - pH (positive effect) had less effect on the response considered with respective coefficients -13.5 and 2.5. Despite that some factors have more effects than others on aaw, none of these factors and their interaction significantly affects aaw. Therefore, all these factors and interactions have negligible effects on aaw.

Table 5. Results of aaw of the gum of B.O

Trials	T (ºC)	pH	Alcohol/water ratio	Observed aaw	Predicted aaw	Residuals
1	50	6	1	480	385.379	94.621
2	85	6	1	270	225.278	44.722
3	50	9	1	700	568.55	131.45
4	85	9	1	526	381.449	144.551
5	50	6	3	466	534.203	-68.203
6	85	6	3	324	379.102	-55.102
7	50	9	3	572	540.374	31.626
8	85	9	3	340	358.273	-18.273
9	67.5	7.5	2	332	323.826	8.174
10	67.5	7.5	2	328	323.826	4.174
11	67.5	7.5	2	330	323.826	6.174
12	38	7.5	2	610	685.865	-75.865
13	97	7.5	2	366	398.107	-32.107
14	67.5	5	2	402	374.729	27.271
15	67.5	10	2	376	511.243	-135.243
16	67.5	7.5	0.3	0	210.157	-210.157
17	67.5	7.5	3.7	418	315.815	102.185

3.6 Validation of the Model

Table 6 present values of the validation criteria elements which were, the AADM and R^2.

Table 6. Elements for validation of the aaw and gums of B.O models

Elements of the validation criteria	Abbreviations	Observed values	Standard values
Correlation coefficient	R^2	82.6344%	100%
Absolute mean deviation analyses	AADM	0.14151155	0

From this data, an R^2 of 82.63% is very far from the standard value, while the AADM had value of 0.14151155 which approaches 0 and allow the validation of this model. The evaluation of the aaw of the obtained gum was done (Figs. 4-5). The surface response of aaw for gums of B.O as a function of the ratio and the pH is thus represented:

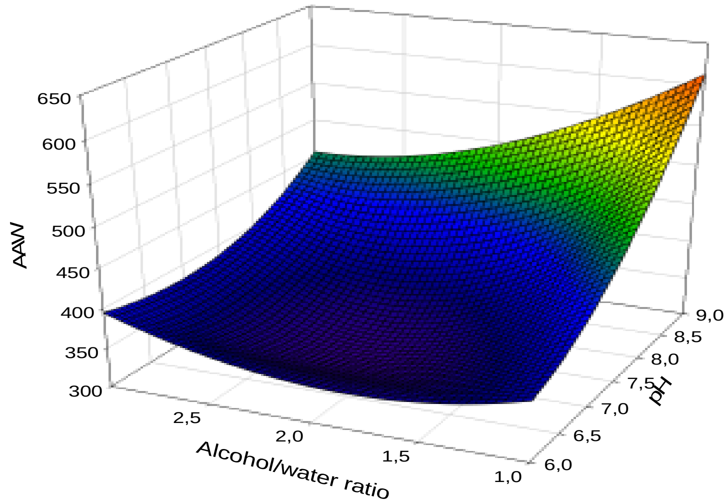

Fig. 4. Evaluation of aaw of the gum of B.O. as a function of the ratio and the pH aaw in g of water/g of gum:: ■ : 0 ; ■ : 200 ; ■ : 400 ; ■ : 600 ; ■ : 800 ; ■ : 1000 ; ■ :1200

Optimum aaw is achieved at pH between 9 and 10, for a ratio between 0.5 and 1 and a temperature of 50ºC. It was observed that aaw evolved with the pH and the alcohol/water ratio. The higher the pH and the ratio in the experimental domain, the more important was the aaw. These conditions of extraction and purification made it possible for the gums to adopt a favourable configuration for water fixation. Moderate temperatures contrary to severe temperatures allow the gum of B.O to preserve their structural integrity during the extraction and to retain a good quantity of water.

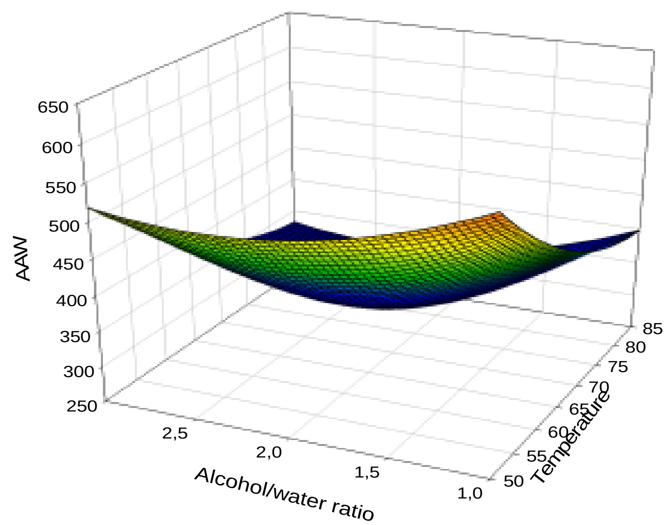

Fig. 5. Evaluation of aaw of the gum of B.O. as a function of the ratio and the temperature;
aaw in g of water/g of gum:■ : 0 ; ■ : 200 ; ■ : 400 ; ■ : 600 ; ■ : 800 ; ■ :1000

3.7 Results of Solubility Index (SI)

The SI determines property of gum. The values obtained were a function of the pH, temperature and the alcohol/water ratio. The predicted values were obtained by simulation of the model (Table 7).

Table 7. Results of the SI of the gum of B.O

Trials	T (℃)	pH	Alcohol/water ratio	Observed SI	Predicted SI	residuals
1	50	6	1	70.51	57.457	13.053
2	85	6	1	75.54	58.743	16.796
3	50	9	1	79.36	62.468	16.891
4	85	9	1	72.22	58.325	13.894
5	50	6	3	71.92	75.233	-3.313
6	85	6	3	66.13	72.440	-6.310
7	50	9	3	72.2	78.415	-6.215
8	85	9	3	67.72	70.192	-2.472
9	67.5	7.5	2	67.35	67.507	-0.157
10	67.5	7.5	2	69.02	67.507	1.512
11	67.5	7.5	2	68.72	67.507	1.212
12	38	7.5	2	75.84	82.877	-7.037
13	97	7.5	2	69.12	77.045	1.512
14	67.5	5	2	68.66	75.584	-6.924
15	67.5	10	2	69.87	77.908	-8.038
16	67.5	7.5	0.3	0	30.952	-30.952
17	67.5	7.5	3.7	71.87	55.880	15.989

From the values obtained, we can postulate that the centre of the experimental domain corresponded to SI values of 68.72, 67.35 and 69.02%; with a standard deviation of 0.89. Experiment 3 had the highest SI and experiment 16 the lowest. With regard to the predicted and the real values, experiments 1, 2, 3, 4, 10, 11 and 17 had observed SI higher than those predicted while experiments 5, 6, 7, 8, 9, 12, 13, 14, 15 and 16 had observed SI lower than those predicted. All these variations were due to experimental errors and the variation in quality of the stocks of ethanol used. Nevertheless, the sum of the residuals was equal to -0.0003; value very close to zero, hence the validation of these results. Experiment 16 presents the lowest SI, while experiment 3 (50ºC, pH 9 and ratio 1) had the best SI which was 79.36. It equally follows from these results that, the gums of B.O. are not completely soluble and their optimum was 79.36 g of water/g of gum.

3.8 The Model Equation

$$Y = 67.8994 - 3.4681X1 + 1.38169X2 + 14.8219X3 + 8.53873X1^2 - 2.715X1\,X2 - 2.04X1\,X3 + 6.26535X2^2 - 0.915\,X2\,X3 - 17.3025X_3{}^2$$

The model equation has a constant of 67.8994. This value corresponds to the average values of the SI obtained. The ratio is the factor which greatly affects the SI because its coefficient is positive and highest (14.8219). The temperature also affects this response with a negative coefficient of -3.4681. The pH had a positive effect on this response with a coefficient of 1.38169 and is thus the factor with the least effect on this response. The interaction temperature – pH and temperature-ratio had as coefficients -2.715 and -2.04

respectively. They thus had a negative influence on the SI. These factors were closely followed by the interaction pH-ratio thus the negative effect is less significant because its coefficient had a small value of 0.915.All these factors and their interactions do not have a significant effect on the SI. This implies that their variation does not have a significant impact on the SI.

3.9 Validation of the Model

Table 8 presents some elements for validation of the model.

Table 8. Elements of validation of the SI and B.O. gums model

Validation elements	Abbreviations	Observed values	Standard values
Determination coefficient	R^2	85.67%	100%
Absolute mean deviation analyses	AADM	0.10447426	0

The R^2 was far from the reference while the AADM had a value of 0.10447426 which was close to zero. Consequently, this model was validated. Surface response (Figs. 6-7) curves for the SI and the gum of B.O. were plotted as a function of the pH and ratio with the Sigma Plot-11 software; the factor temperature was fixed at the centre of the experimental domain.

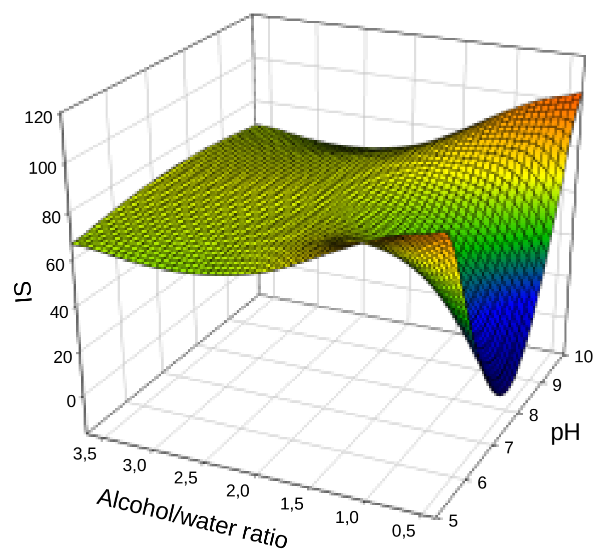

Fig. 6. Evaluation of SI of the gum of B.O.as a function of the pH and alcohol/water ratio SI:■ : 0% ; ■ : 20% ; ■ : 40% ; ■ : 60% ; ■ : 80% ; ■ : 100% ; ■ :120%

The SI is highest at pH 6 and 9, an alcohol/water ratio between 0.5 and 1 and at temperature of 60ºC and above 90ºC. Indeed, the SI was inversely proportional to the alcohol/water ratio and the optimum pH was 6 and 9. This is an indication that, when the gums of B.O. are extracted at moderate temperature at pH 6 or 9 and precipitated with a low volume of ethanol in the experimental domain, they adopt a configuration which facilitates their solubility in aqueous medium. Knowing that the solubility of gums is related to the solute/solute interactions and solute/water interactions competition, these optimum conditions would permit the solute/solute interactions to be lesser than the solute/water interactions; thus an increase in the solubility of the gum of B.O. It is important to note that the solubility of the gum of B.O. is optimum out of the neutrality zone.

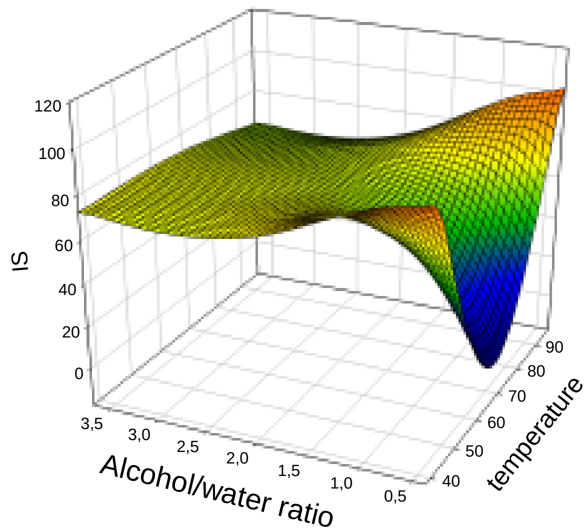

Fig. 7. Evaluation of the SI of the gum of B.O. as a function of the alcohol/water ratio and the temperature; SI:: ■ : 0% ; ■ : 20% ; ■ : 40% ; ■ : 60% ; ■ : 80% ; ■ : 100% ; ■ :120%

3.10 Results of the Emulsifying Activity

The EA of the gum of B.O. was determined as a function of temperature, pH and the alcohol/water ratio as presented in Table 9. The predicted values and the residuals are also included in the table.

Table 9. Results of the EA of the gum of B.O

Trials	T (℃)	pH	Ratio Alcohol/water	Observed EA	Predicted EA	Residuals
1	50	6	1	32.5	27.432	5.4574
2	85	6	1	30	32.743	-2.743
3	50	9	1	35	29.542	5.457
4	85	9	1	25	18.603	6.396
5	50	6	3	45	48.275	-6.925
6	85	6	3	65	67.335	-2.335
7	50	9	3	55	52.489	2.510
8	85	9	3	50	51.945	-1.945
9	67.5	7.5	2	47.5	46.414	1.085
10	67.5	7.5	2	47.5	46.414	1.085
11	67.5	7.5	2	45	46.414	-1.414
12	38	7.5	2	37.5	43.793	-6.293
13	97	7.5	2	52.5	50.622	1.878
14	67.5	5	2	55	51.541	3.459
15	67.5	10	2	32.5	40.374	-7.874
16	67.5	7.5	0.3	0	6.925	-6.925
17	67.5	7.5	3.7	55	49.135	5.865

The results of the EA revealed that, the experiments at the centre of the experimental domain corresponded to the following values: 45, 47.5, and 47.5 %; with a standard deviation of 1.44. Moreover, experiments 1, 3, 4, 7, 9, 10, 13, 14 and 17 had values of the observed EA higher than that predicted while experiments 2, 6, 8, 11, 12, 15, and 16 had real values lower than those predicted. All these variations were due to experimental errors. The sum of the residuals was equal to -0.000005, a value very close to 0; thus, the results can be validated. The lowest EA was obtained with experiment 16 which was carried out under the conditions: temperature 67.5ºC, pH 7.5 and ratio 0.3. The best EA was obtained with experiment 6 and corresponded to a temperature of 85ºC, a pH of 6 and a ratio of 3. It was noticed that the emulsifying activity was best under the optimum conditions of yield. Therefore the higher the concentration of the gum the better the emulsifying activity. Indeed the emulsifying activity of gums is related to an increase in the viscosity of the medium which prevents molecular motion. Thus the optimum extraction of gums is strongly related to a high emulsifying activity. Due to emulsifying property, these gums can be used to formulate emulsions such as mayonnaise and creams.

3.11 Equation of the Model

$$Y = 46.4142\ 3 + 4.06051X_1 - 6.63994X_2 + 27.0924X_3 + 0.560982\ X_1^2 - 8.125X_1\ X_2 + 6.875X_1X_3 - 0.3229_1^2X_2^2 - 0.625X_2\ X_3 - 11.8134X_3^{\mathbf{2}}$$

The average values of the EA obtained was 46.41423 which corresponded to the constant of the equation The EA is highly affected by the alcohol/water ratio because this factor has a positive coefficient of 27.0924. It is followed by the pH factor, thus the negative effect is evaluated by the value of its coefficient which amounted to -6.63994. The temperature had a positive effect (coefficient, 4.06051), which was less significant than the other factors. The quadratic interaction temperature-temperature had a negative impact with a coefficient of -11.8134 being highest for the interactions. Temperature-pH interaction with a negative coefficient of -8.125 closely followed, then interaction temperature-ratio with a positive coefficient of 6.875. Interactions of pH-ratio and pH-pH had negative effects and lowest contribution with coefficients -0.625 and - 0.322912 respectively.

It was only the factor alcohol/water ratio which had a highly significant effect on the EA. The quadratic interaction ratio-ratio had a significant effect too. The remaining factors and the interactions did not have a significant effect on the EA. Thus only the variations of the ratio had an impact on the EA.

3.12 Validation of the Model

The model validation elements particularly the R^2 and the AADM which are used for error rate estimations are summary in Table 10.

Table 10. Validation elements for the EA and gum of B.O. model

Validation elements	Abbreviation	Observed values	Standard values
Determination coefficient	R^2	91.0568 %	100%
Absolute mean deviation analyses	AADM	0.0908799	0

In this case the R^2 was 91.0568%, while the AADM had a value of 0.0908799 which is very close to 0; hence allow the validation of this model. The surface response of the EA was plotted as a function of the pH and the alcohol/water ratio with the temperature being fixed at the centre of the experimental domain (Figs. 8-9).

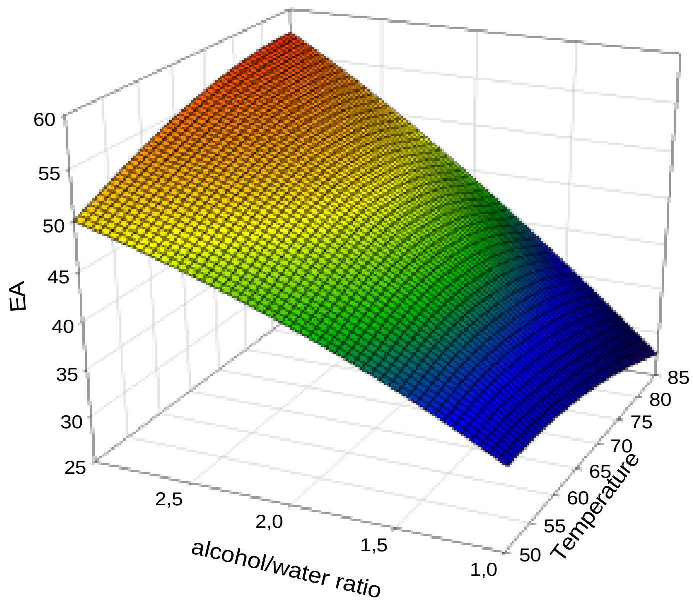

Fig. 8. Evaluation of the EA and the gum of B.O. as a function of alcohol/water ratio and temperature; EA : ■ : 0 %; ■ : 10% ; ■ : 20% ; ■ : 30 %; ■ : 40% ; ■ : 50% ; ■ : 60% ; ■ :70%

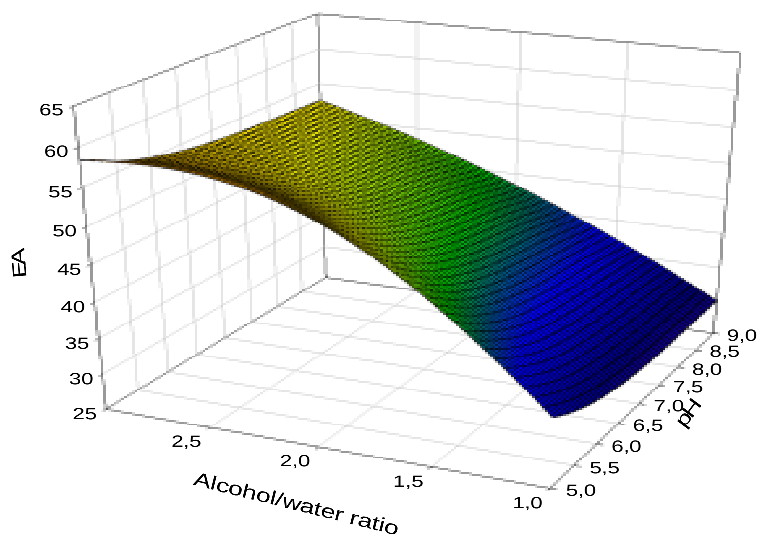

Fig. 9. Evaluation of the AE of the gum of B.O. as a function of the alcohol/water ratio and pH; EA : ■ : 0 %; ■ : 10% ; ■ : 20% ■ : 30 %; ■ : 40% ; ■ : 50% ; ■ : 60% ; ■ :70%

The emulsifying activity is optimum with pH ranging from 5 to 9, with alcohol/water ratios higher than 2.5 and at temperatures higher than 70ºC. This implies that pH variation in the experimental domain had very little effect on the EA. On the other hand, EA is optimum starting from a ratio of 2.5 at a high temperature. These conditions thus produce gums of high viscosity which prevents free motion of molecules, hence favouring the formation of emulsions [13,14,15]. It was observed that, these conditions corresponded to the conditions of optimum extraction; implying that the greater the concentration of gums the higher the emulsification activity.

3.13 Determination of the Optimum Points

With the application of the methodology of surface response in this study, one of the objectives was to determine the compromise zone within the experimental domain. Taking into account the fact that it is easy to have a combination of factors permitting the accessibility of optimal responses for all factors at the same time, the results obtained were used to determine the acceptable compromise zone (Table 11).

Table 11. Determination of the optimum zone of the gum of B.O

Factors	yields	aaw	SI	EA	Optimum
pH	5 -6	9 - 10	6 - 9	5 - 9	7.3
Ratio	3 – 3.5	0.5- 1	0.5 - 1	>2.5	3.0
Temperature (ºC)	80 - 100	50	60	70	67.5

From this table, the optimum G.B.O. is obtained at a temperature of 67.5ºC, a pH of 7.3 and alcohol/water ratio of 3.

4. CONCLUSION

The optimisation of the extraction of gums from B.O. was the main focus in this work. It was revealed that the factors pH and alcohol/water ratio had significant effects on the extraction of the gums of B.O. Indeed, the gums of B.O. were extracted in an optimum manner at a pH of 7.3, alcohol/water ratio of 3 and a temperature of 67.5ºC. Despite the low efficiency values, gums obtained offered interesting functional properties exploitable in food industry.The optimisation of the extraction of gums considered was thus carried out; however, the study of the effect of other factors could contribute to improve the optimisation of the extraction yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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