British Journal of Applied Science & Technology
4(35): 4860-4877, 2014
ISSN: 2231-0843

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Optimization of the Extraction of Pectin from *Cucumis melo*

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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, TAE and SLA managed the literature searches, analyses of the study. Author CMM supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2014/13441
Editor(s):
(1) Teresa De Pilli, University of Foggia, Department of Science of Agriculture of Food of Environnement (SAFE), Via Napoli, 25; 71100 Foggia, Italy.
Reviewers:
(1) Anonymous, University of Misurata, Libya.
(2) Preeya Puangsomlee, Biology, khon Kaen University, Thailand.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=691&id=5&aid=6273

Original Research Article

Received 17th August 2014
Accepted 12th September 2014
Published 29th September 2014

ABSTRACT

Cucumis melo, a plant found in Cameroon, is being used at the artisanal scale as vegetable in some Cameroon local dishes. These extracts are still to be exploited at the industrial level. In order to add more value to the Cameroon vegetative potential in general, the optimisation of pectin extraction from this plant was studied. Pectin was extracted from the fruits of C. M. by solubilisation in aqueous media followed by ethanol purification. A centred composite experimental design with three factors: temperature, pH

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and alcohol/water ratio was used to this effect. Responses considered were, water absorption capacity, solubility index and the emulsifying activity. The results obtained were analysed with the Statistica Centurion Software and the validation criteria for the obtained models where: the AMDA[1], the determination coefficient R^2 and the sum of residuals. Sigma plot-11 software was used to plot surface response curves so as to better visualise the results. From this work, it follows that the optimum conditions for the extraction of pectin from *Cucumis melo*, was attained at pH 2.4, alcohol/water ratio of 1.5 and a temperature of 74°C.

Keywords: Food pectin; Cucumis melo; extraction; optimisation.

1. INTRODUCTION

Generally, many plants serve as a source of raw material for the manufacture of water-soluble gums. These substances are exploited in the cosmetic, therapeutic, textile and food industries amongst others. 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 [1,2]. Amongst several studies, proved the presence of pectin in the fruits of *Cucumis melo,* commonly called *African melon*, and demonstrated the presence of pectin in some vegetative species of Africa [2,3,4], the industrial exploitation of these resources are still rudimentary in Cameroon; indeed, this plant is only used for human consumption [5,6]. In order to promote the industrial application of this local plant, this work had as main objective to optimise the extraction processes and the characterisation of pectin obtained from the fruits of *Cucumis melo*.

Otherwise, pectin has 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 pectin extraction vary according to their chemical composition. Non ionised pectin resulting from fruits is more soluble at a pH range from 6 to 9. As for their extraction temperature, it varies between 50 and 85°C, in order to limit degradations due to severe heat treatment [7]. For pectin, 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 100°C [8]. 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 pectin. Indeed being an organic solvent, alcohol facilitates the precipitation of polysaccharides which are insoluble in organic solvents [9]. However, the quantity of pectin precipitated depends strongly on the alcohol/water ratio and the interactions between the various components of the raw material during the purification of pectin, hence the importance of this factor in pectin extraction. Generally pectin extraction depends on the temperature, pH and alcohol/water ratio for which pectin extracted have different yields and properties; it is the reason to study how these factors affect the extraction of water soluble pectin form the fruit of *Cucumis melo*.

[1] *Absolute Mean Deviation Analyses*

2. MATERIALS AND METHODS

2.1 Sampling of the *Cucumis melo* (C.M.) Fruits

Mature ripe fruits of *C M*. were purchased at Makenene, a town located in the Centre Region of Cameroon in July 2012. They were transported to the Physico-Chemistry Laboratory of ENSAI, University of Ngaoundere in polyethene bags. Once at the laboratory, these fruits were washed with tab water and peeled. The Pulp obtained was sliced into 1 mm thick lamellas with the help of a knife. These lamellas were blanched in distilled water at 80°C for 5 minutes, then dried at 45°C for 72 hours with an electric dryer (P. Dominioni Lurate Caccivio Como, Italy). After drying, a cereal crusher (SAMAP) was used to crush the dried lamellas into a powder. This powder was sieved with a 400 µm mesh sieve, and preserved in an airtight container prior to analysis.

2.2 Extraction of Pectin from C. M.

The method used for extraction was the extraction in aqueous acid. The precipitation was with ethanol 95°C. The wet pectin obtained were then dried with an electric dryer and preserved in polyethylene bags. In a more detailed approach, the various stages for the extraction process of C. M. pectin was as follows:

2.2.1 Preparation of the extraction solution

10 g of sample was weighed out on an analytical balance (Denver Instrument, model Apx-3202) and each dispensed into 250 ml distilled water, to obtain a solution of final concentration of 10g/250 ml. In order to optimise the extraction of pectin, a centre composite experimental design was used to permit the variation of pH within experimental limits. *A* pH range from 1.5 to 4 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 from 60 to 100°C.

2.2.2 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 extraction optimisation. Solutions at different temperatures were placed under agitation at a speed of 3600 rpm for 4 hours.

2.2.3 Centrifugation

After extraction, the solutions were centrifuged at 3600 rpm for 15 minutes. The supernatant was recovered and stored at 4°C, while the sediments were discarded. The resulting supernatants from the two successive extractions were considered to be the purified pectin.

2.2.4 Purification

After measurement of the supernatant volume, precipitation was carried out by incorporating a given volume of ethanol at 95°C into the medium. The alcohol/water ratio was between 1 and 3 and the precipitation time was fixed at 30 minutes. This operation was optimised by

the use of a centred composite experimental plan, whose matrix is presented in the Table 2. After precipitation, the pectin was thus separated by filtration. They were washed with ethanol in order to eliminate the impurities.

Table 1. *Cucumis melo* pectin's extraction matrix

Trials	Temperature (°C)	pH
1	60	1.5
2	90	1.5
3	60	4
4	90	4
5	60	1.5
6	90	1.5
7	60	4
8	90	4
9	75	2.75
10	75	2.75
11	75	2.75
12	50	2.75
13	100	2.75
14	75	0.65
15	75	5
16	75	2.75
17	75	2.75

P.S: During the handling, the pH of 0.647 was replaced with pH 1 to practical convenience

Table 2. *Cucumis melo* pectin's purification matrix

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

2.2.5 Drying

The pectin was dried at 38°C for 15 hours with an electric dryer. They were then crushed in order to obtain a powder which was conditioned in airtight packages. Fig. 1 illustrates the extraction method.

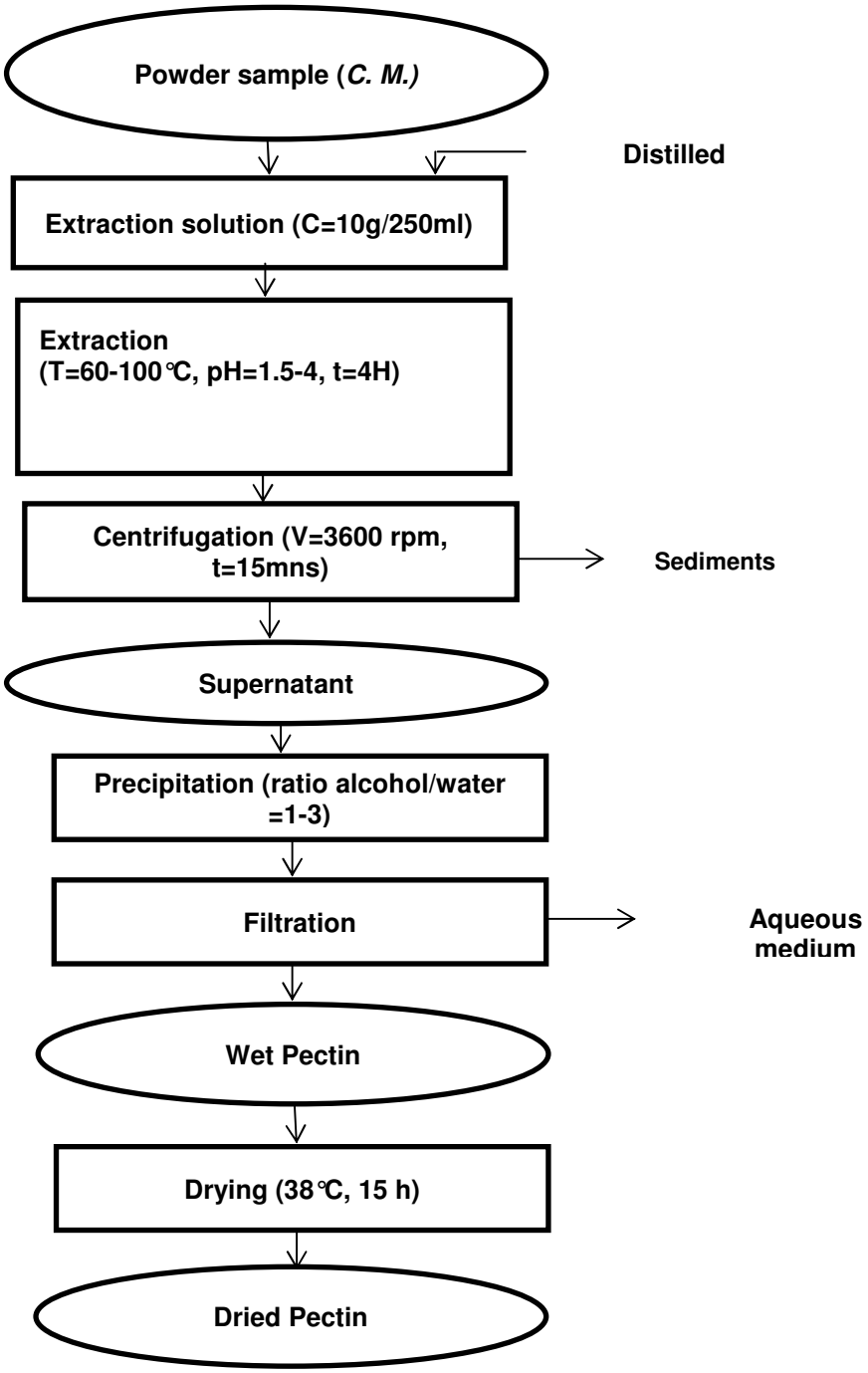

Fig. 1. Extraction process for the pectin of *Cucumis melo*

2.3 Determination of the Extraction Yield

The extraction yield (Y) determines the proportion or the percentage of pectin 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 pectin

2.4 Determination of the Absorbency Apparent Water (aaw)

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

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

2.5 Determination of the Solubility Index

The solubility index (SI) expresses the percentage of dissolved pectin 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. [7], 0.1 g of pectin was added to 0.4 g of corn starch and the powder mixture was dissolved into 10 ml of water and agitated for 30 mins. The homogenate was centrifuged at 5600 turns/min for 30mins. The sediments m_2 was recovered, weighed and dried at 105℃ in an oven for 24 h. The weight of the dry sediments (m_3) was determined and the solubility index was thus determined:

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

2.6 Determination of the Pectin Emulsifying Properties

The emulsifying properties of pectin determine its capacity to permit 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.

2.6.1 Preparation of the emulsions

0.1 g of pectin was dissolved in 5 ml of water, and then mixed with 5 ml 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.

2.6.2 Estimation of the emulsifying activity (EA)

The emulsifying activity was evaluated by the method adapted from Muschiolik [11]. 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 pectin emulsification activity (AE) in percentage was determined thus:

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

With**:** He; the height of the emulsified layer and Hw; the total height of the liquid in the tube.

3. RESULTS AND DISCUSSION

3.1 The Extraction Yield

The yields of the extraction of pectin from C. M. were obtained as a function of the factors: temperature, pH and alcohol/water ratio. Table 3 presents the observed, the predicted yields and the calculated residuals.

Table 3. Results of the extraction yield of pectin from C. M.

Trials	Temperature (°C)	pH	Alcohol/water ratio	Observed yields	Predicted yields	Residuals
1	60	1.5	1	4.53	3.359	1.170
2	90	1.5	1	3 .8	3.447	0.352
3	60	4	1	0.66	0.289	0.370
4	90	4	1	0.9	0.554	0.345
5	60	1.5	3	1.8	1.613	0.186
6	90	1.5	3	2.486	2.324	0.161
7	60	4	3	0.23	0.050	0.179
8	90	4	3	0.3	0.938	-0.638
9	75	2.75	2	0.82	0.774	0.045
10	75	2.75	2	0.8	0.774	0.025
11	75	2.75	2	0.833	0.774	0.058
12	50	2.75	2	0.406	1.283	-0.877
13	100	2.75	2	2.23	2.104	0.125
14	75	0 .65	2	3.16	4.016	-0.856
15	75	5	2	0.373	0.269	0.103
16	75	2.75	0.3	0.24	1.3152	-1.0752
17	75	2.75	3.7	0.493	0.170	0.322

From these results it arose that the centre experiments had values of 0.82, 0.8, and 0.833%; with a standard deviation of 0.016. It can be observed that experiments 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 15 and 17 had experimental values higher than those predicted while experiments 8, 12 and 16 had observed yields lower than the predicted one. This could be due to experimental errors and variations in the quality of reagents used. Moreover the sum of the residuals amounted to 0.0000026 which is very close to zero. Results of the C. M pectin yields were thus valid. The lowest yield value obtained occurred with experiment 7

and this corresponded to the following experimental conditions: temperature of 60°C, a pH of 4 and a ratio of 3. The optimum was attained at experiment 11 corresponding to a temperature of 60°C, a pH of 1.5 and a ratio of 1.

However, it can be observed that, this yield (4.53%) was lower than 7% as observed by Ptichkina et al. [8]. These results can be justified by the state of the maturity of the fruit and the extraction time. Indeed, the fruits used for this study were at the ripening stage, whereas it is advisable to extract pectin from mature fruits which are not sufficiently ripe since high pectinase activity occurs in ripe fruits. Moreover, the extraction time was fixed at 4 hours, a time which could have been sufficiently long to promote the hydrolysis of pectin. Indeed, the extraction time of pectin depends on the maturity of the raw material.

3.1.1 Equation of the model

$$Y = 0.77463 + 0.488191X_1 - 2.22792X_2 - 0.680758X_3 + 0.650268X_1^2 + 0.0885X_1X_2 + 0.3115X_1X_3 + 0.967409X_2^2 + 0.7535X_2X_3 - 0.0225412X_3^2$$

With: X_1 = Temperature; X_2 = pH; X_3 = Alcohol/water ratio

From this model, the average of the extraction yields of pectin was 0.77463 which corresponded to the constant of the model. The pH factor had a greater impact on pectin extraction from C. M. since it had the highest coefficient - 2.22792; indeed its impact was negative on this response. It was closely followed by the factor ratio which also had a negative effect and thus the coefficient was - 0.680758.Thetemperature factor had a positive effect which was less significant than the others factors, with a coefficient of 0.488. The pH-pH interaction had a positive effect which was more significant. It was followed by the pH-ratio, temperature-temperature and temperature-ratio interactions, which had coefficients of 0.967409, 0.7535, 0.650268, and 0.3115 respectively.

According to the variance analysis, only the pH had a highly significant effect on the extraction yield of pectin from C. M. the other factors and their interactions did not show any marked significant effect on this response. However, the R^2 was 85.7579%; the AMDA was 0.131 Table 4 with the AMDA close to 0 this model was validated.

Table 4. Validation of the yield model for the extraction of the pectin from C. M.

Validation elements	Abbreviations	Observed values	standard values
Determination coefficient	R^2	85.7579 %	100%
Absolute mean deviation analyses	AMDA	0.13138215	0

In order to visualise the effect of the various factors on the extraction yield for pectin of C. M, the surface response curve was plotted Figs. 2 and 3 by considering the factors which had a greater effect on the yield; the temperature was fixed at a value within the experimental limits.

From these surface responses, the yield of extraction of pectin from C. M. was highest at an alcohol/water ratio of 1, a temperature of 60°C and a pH of 1.5. This illustrates that low temperatures combine with low pH and small ratios in the experimental domains permitted a better extraction of pectin from C.M. In fact, pectin being made up of mainly galacturonic acid residues would easily be solubilised in acidic medium because under these conditions their affinity for the medium becomes dominating. The moderate temperatures would allow

the maintenance of their structural integrity contrary to severe temperatures which combined with an acid pH lead to the hydrolysis of pectin. With regard to the ratio, it was observed that the yield was inversely proportional to the ratio up to a threshold ratio of 1. This is justified by the composition of pectin. The latter is very rich in galacturonic acid whose acidic groups can form ester bonds with ethanol in the presence of abundant ethanol in the medium. This justifies a reduction of the yield with an increase in ethanol volume.

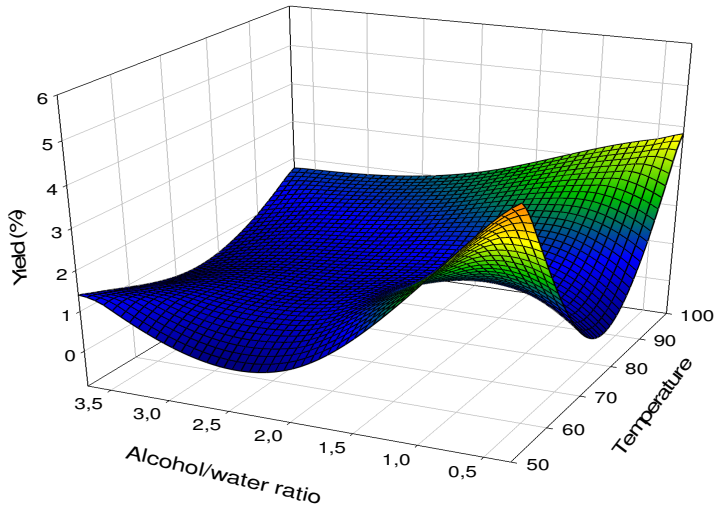

Fig. 2. Evaluation of the yield of the extraction of pectin from C. M. as a function of the alcohol/water ratio and the temperature: Yield: ■ : 0 %; ■ : 1% ; ■ : 2% ; ■ : 3%; ■ : 4% ; ■ : 5% ; ■ :6%

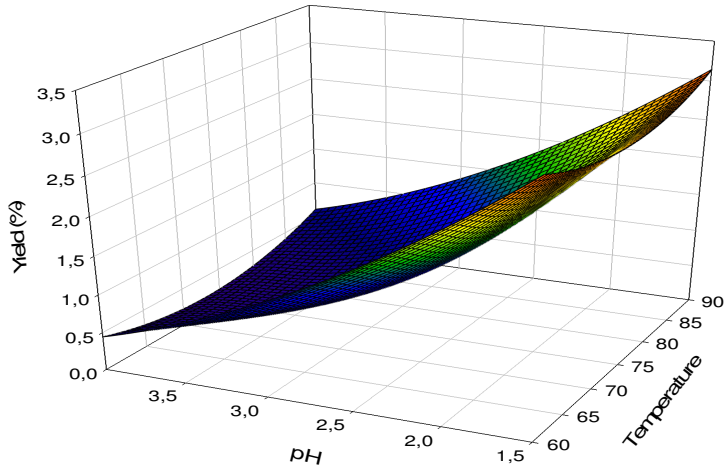

Fig. 3. Evaluation of the yield of the extraction of pectin from C. M. as a function of alcohol/water ratio and the pH Yield: ■ : 0 %; ■ : 2% ; ■ : 4%; ■ : 6% ; ■ : 8% ; ■ :10%

3.2 Absorbency Apparent Water (aaw)

Absorbency apparent water (aaw) was determined as a function of the factors: temperature, pH and alcohol/water ratio. Table 5 thus contains observed, predicted and the values of the residuals of aaw.

Table 5. Results of aaw of the pectin of C.M.

Trials	Temperature(°C)	pH	Ratio alcohol/water	Observed aaw	Predicted aaw	Residuals
1	60	1.5	1	36	18.275	25.224
2	90	1.5	1	34	5.404	28.595
3	60	4	1	10	33.567	-23.567
4	90	4	1	58	99.195	-41.195
5	60	1.5	3	128	128.159	-0.159
6	90	1.5	3	78	95.787	-17.787
7	60	4	3	19	88.950	-69.950
8	90	4	3	76	135.079	-59.079
9	75	2.75	2	100.1	105.378	-5.278
10	75	2.75	2	102	105.378	-3.378
11	75	2.75	2	104	105.378	-1.378
12	50	2.75	2	46	20.775	25.224
13	100	2.75	2	82	48.740	33.259
14	75	0 .65	2	42	78.808	-36.808
15	75	5	2	220	124.707	95.293
16	75	2.75	0.3	25	33.9707	-8.970
17	75	2.75	3.7	224	156.545	67.455

From this table, the aaw of the centre experiments were: 100.1, 102 and 104 g water/g of pectin; with a standard deviation of 1.95. The results revealed that the observed aaw were higher than predicted aaw for experiments 2, 4, 10, 11 12 and 15 and that the real values were lower than the predicted values for experiments 1, 3, 5, 6, 7, 8, 9, 13, 14, 16 and 17. This could be a result of experimental errors. In addition, the sum of the residuals was 0.00021a value very close to zero. Hence, the model can thus be validated. The optimum was obtained with experiment 15 which corresponds to operation conditions of temperature 75°C, pH 2.75 and ratio 3.68. These combinations of factors favour the acquisition of a favourable configuration of pectin for water absorption. Despite these, some values of aaw were lower than the aaw of the blank sample, the optimum value of aaw obtained was 224 g of water/g pectin which is largely higher than aaw of the blank sample. The pectin of C. M. can thus be used in bread making, in order to improve dough consistency.

3.2.1 Equation of the model

$$Y = 105.378 + 16.6284X_1 + 27.2917X_2 + 72.8831X_3 - 49.9357X_1^2 + 39.25X_1X_2 - 9.75X_1X_3 - 2.55988X_2^2 - 27.25X_2X^3 - 7.1561X_3^2$$

With: X_1 = Temperature; X_2 = pH; X_3 = Alcohol/water ratio

The equation constant, which is the average aaw is 105.378. We can observe that, the factor ratio has the greatest effect on aaw followed by the pH and the temperature with positive effects on this response. The quadratic interaction temperature-temperature had the most

negative effect on aaw with a coefficient of -49.935. It is followed by the interaction temperature-pH with a positive effect and a coefficient of 39.25; the interaction pH-ratio with a negative effect and -27.25 as coefficient. Finally, the interaction temperature-ratio has a negative effect with -9.75 as a coefficient. The quadratic interactions ratio-ratio and pH-pH were least with negative effects and coefficients of -7.1561 and -2.55988 respectively. However all these factors and interactions do not have a significant effect on aaw as shown by the variance analysis. Indeed, the variations of all these factors as well as their interactions do not have a considerable influence on aaw for the pectin of C. M. The validation of the aaw model was done by evaluating the R^2 and the AMDA Table 6.

Table 6. Validation of the aaw model for pectin of C.M.

Validation elements	Abbreviations	Observed values	Standard values
Determination coefficients	R^2	83.98%	100%
Absolute mean deviation analyses	AMDA	0.31242399	0

The R^2 had a value of 83.98% and is thus distant from the standard value. The AMDA was equal to 0.31242399. This value being closed to zero permitted the validation of this model. The surface response of aaw of pectin of C.M. Figs. 4 and 5 could be represented as a function of the pH and the ratio. The temperature was fixed within the experimental domain.

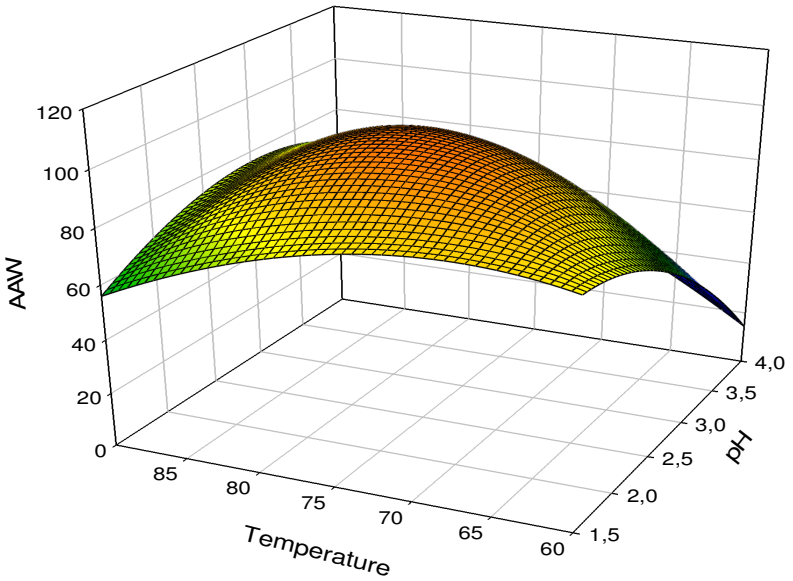

Fig. 4. Evaluation of aaw of the pectin of C.M. as a function of temperature and pH: aaw in g water/g pectin: : 2 ; : 0 ; : 100 ; : 200; :300

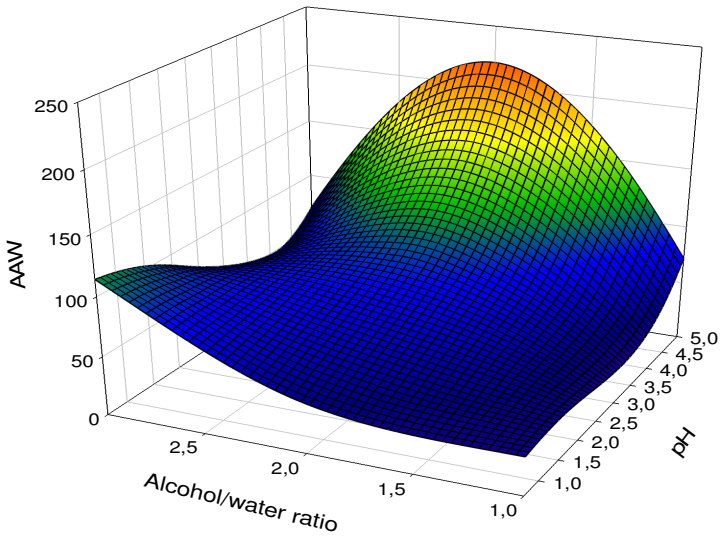

Fig. 5. Evaluation of aaw of the pectin of C. M. as a function of alcohol/water ratio and the pH aaw in g water/g pectin: : 0 ; : 50 ; : 100 :150; : 200; :250

From these Figures, we can see that aaw is optimum on two levels; firstly at a pH range from 4 to 4.5 for a ratio from 1.5 to 2.5; and secondly at a pH range between 2 and 3.5 for a ratio greater than 3. These conditions impart a favourable configuration to pectin, hence promoting great water adsorption. From these surface curves, it also arises that the interaction pH-ratio had a dominating effect on aaw for pectin of C. M. and that the two optimum intervals of pH correspond to precise intervals of alcohol/water ratio.

3.3 Solubility Index (SI)

The SI obtained according to temperature, pH and alcohol/water ratio is presented on Table 7. This table also contains the predicted SI and the calculated residuals from the actual and predicted values.

This Table presents experiments at the centre with values being 53.95, 56.11 and 55.10 with a standard deviation of 1.080. The observation of the experimental and predicted values show that the experimental SI are higher than the predicted one for experiments 1, 5, 7, 10, 13, 15 and 17 while they are lower than the predicted one for experiments 2, 3, 4, 6, 8, 9, 11, 12, 14 and 16. These variations are caused by the experimental errors and the use of different ethanol stocks. Nevertheless, these results can be validated because the sum of the residuals obtained through the differences between the actual values and the predicted values is 0.0000014which is very close to 0. The lowest SI is 47.02% and corresponds to experiment 3 (60°C, pH 4 and ratio 1).The highest SI is 65.82% and corresponds to a temperature of 75°C, a pH of 5 and an alcohol/water ratio of 2 (experiment 15).

3.3.1 Equation of the model

$$Y = 55.4672 - 2.3247X_1 + 4.87747X_2 + 8.05757X_3 - 4.1371X_1^2 + 8.53X_1X_2 + 1.735X_1X_3 - 1.83198X_2^2 - 4.46X_2X_3 - 2.37291X_3^2$$

With: X_1 = Temperature; X_2 = pH; X_3 = Alcohol/water ratio

Table 7. Results of the SI of the pectin of C.M.

Trials	Temperature (°C)	pH	Alcohol/ water ratio	Observed SI	Predicted SI	Residuals
1	60	1.5	1	49.9	48.893	1.006
2	90	1.5	1	34.45	36.303	-1.853
3	60	4	1	47.02	49.701	-2.681
4	90	4	1	47.22	54.171	-6.951
5	60	1.5	3	61.51	59.676	1.833
6	90	1.5	3	48.12	50.556	-2.436
7	60	4	3	49.1	45.335	3.764
8	90	4	3	53.38	59.503	-6.123
9	75	2.75	2	53.95	55.467	-1.517
10	75	2.75	2	56.11	55.467	0.642
11	75	2.75	2	55.10	55.467	-0.367
12	50	2.75	2	50.95	51.571	-0.621
13	100	2.75	2	55.52	47.661	7.858
14	75	0 .65	2	47.17	48.775	-1.605
15	75	5	2	65.82	56.977	8.842
16	75	2.75	0.3	48.3	51.563	51.563
17	75	2.75	3.7	62.36	58.887	3.473

The average of the SI of the pectin of C.M. corresponds to the constant of the model 55.4672. The factor ratio had the greatest positive effect on the SI with coefficient of 8.0575. It was followed by the positive effect of the pH and the negative effect of the temperature with coefficients of 4.87747 and -2.3247 respectively. The interaction temperature-pH had the greatest positive effect on the SI. It was followed by the ratio-pH interaction having a negative coefficient of -4.46and the negative temperature-temperature interaction with a coefficient of -4.46. The quadratic interaction ratio-ratio had a negative effect with a coefficient of -2.37291 and finally the interaction temperature-ratio of a positive effect with a coefficientof1.735.

It is important to note that all these factors and interactions did not have a significant effect on the SI of the pectin of C.M. as shown by the variance analysis. The solubility indices for the pectin of C. M. obtained permitted the establishment of a model. The validation criteria of this model are presented in Table 8 which contains the values of R^2 and the AMDA.

Table 8. Validation of the model of the SI for the pectin of C. M.

Validation elements	Abbreviation	Observed values	Standard values
Coefficient de correlation	R^2	86.1442%	100%
Absolute mean deviation analyses	AMDA	0.06127348	0

From this table, we can see that R^2 is different from the standard value. With regard to the AMDA, it is 0.06127348 values very close to 0. The model of the IS for the pectin of C.M. was thus validated. The result obtained was used to plot surface response curves Figs. 6 and 7. This curve represents the SI as a function of the pH and of the alcohol/water ratio; temperature was fixed at its central value.

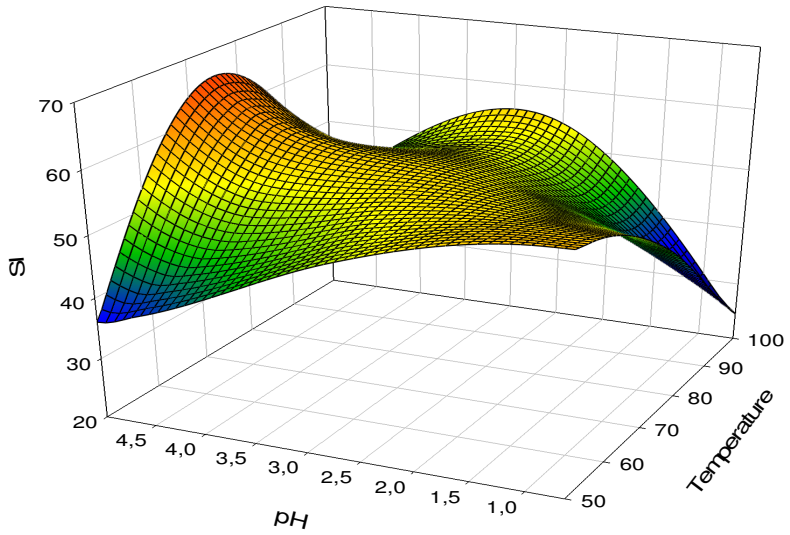

Fig. 6. Evaluation of the SI of the pectin of C.M. as a function of the pH and the temperature SI: :20% ; : 30% ; : 40% ; : 50% ; : 60%; :70%

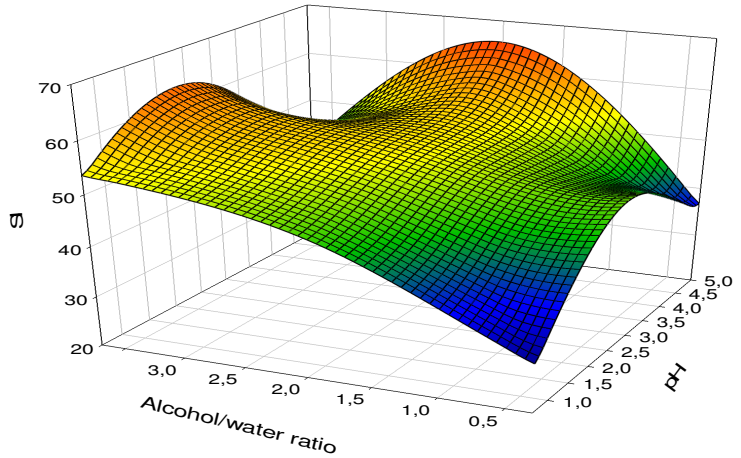

Fig. 7. Evaluation of the SI of the P.C.M. as a function of the pH and the alcohol/water ratio SI: : 35% ; : 40% ; : 45% ; : 50% ; : 55% ; : 60%; : 65%; :70%

These Figures show that, the SI was optimum at pH values higher than 4 within the experimental limits, with an alcohol/water ratio range from 1.5 to 2.5 and temperature between 70 and 80°C. The Solubility is related to the competition between the solute-solute interactions and water -solute interaction. These conditions of extraction would favour the solute-water interaction than the solute-solute interactions. Moreover knowing that the solubility of pectin depends on their ionic state and that of the saline form is more soluble than the acid form, it is thus normal that the SI was highest at higher pH within the experimental limits. With the ideal being SI of 100%, the must thus undergo an additional treatment so that there SI is improved.

3.4 Emulsifying Activity (EA)

The EA of the pectin of C.M. was evaluated according to the various factors chosen within the framework of this study; the results obtained Table 9 were the observed and the predicted AE.

Table 9. Results of the AE of the pectin of C.M.

Trials	Temperature (°C)	pH	Alcohol/water ratio	Observed AE	Predicted AE	Residuals
1	60	1.5	1	60	66.7847	-6.7847
2	90	1.5	1	50	58.7072	16.9687
3	60	4	1	50	33.0313	16.9687
4	90	4	1	40	44.1573	-4.1573
5	60	1.5	3	52.94	59.6343	-6.6943
6	90	1.5	3	77.77	75.2958	2.4742
7	60	4	3	41	64.2018	-23.2018
8	90	4	3	95	94.4483	0.5517
9	75	2.75	2	88.85	89.3842	-0.5042
10	75	2.75	2	88.8	89.3842	-0.5042
11	75	2.75	2	88.88	89.3842	-0.5042
12	50	2.75	2	94.73	70.7471	23.9829
13	100	2.75	2	52.63	67.798	-15.168
14	75	0 .65	2	44.44	50.9911	-6.5511
15	75	5	2	75.67	60.304	15.366
16	75	2.75	0.3	40	39.5388	0.4612
17	75	2.75	3.7	97.29	84.3178	12.9722

The centre experiments had AE values of 88.88, 88.85 and 88.8; with a standard deviation of 0.04. The actual values and those predicted showed that experiments 3, 6, 8, 12, 15, 16 and 17 had observed EA higher than those predicted and that of experiments 1, 2, 4, 5, 7, 9, 10, 11, 13 and 14 had observed EA lower than those predicted. These differences could be attributed to experimental errors. The sum of the residual was 0.0001 which is very closer to 0; hence these results were reliable and validated. The lowest EA was 40% and corresponded to experiments 4 and 16. The highest EA was 97.29% and corresponded to experiment 17 (75°C, pH 2.75 and ratio 3.7). Due to the emulsifying capacity of this pectin they could be applied in production of many products such as mayonnaise and creams.

3.4.1 Equation of the model

$$Y = 89.3842 - 1.75345X_1 + 5.53749X_2 + 23.8796X_3 - 14.221X_1^2 + 7.2925X_1X^2 + 24.7075X_1X_3 - 23.8554X_2^2 + 6.3225X_2X_3 - 17.7814X_3^2$$

With: X_1 = Temperature; X_2 = pH; X_3 = Alcohol/water ratio

This equation has as a constant 89.3842; this value corresponds to the sum of the EA obtained. The ratio had the greatest effect on the EA and this effect was positive. It was closely followed by the pH with a coefficient of 5.53749and temperature with a negative coefficient of -1.75345. The interactions temperature-ratio, Temperature-pH and pH-ratio had a significant and positive impact on the EA of the pectin with the interaction temperature-ratio having the greatest effect with 24.7075 as coefficient. It was followed by the quadratic interaction pH-pH which has a negative effect with a coefficient of -23.855. Then, the

quadratic interaction temperature-temperature with a negative effect, followed by the interaction temperature-pH with a coefficient of 7.2925 (positive effect) and finally by the interaction pH-ratio of coefficient 6.3225(positive effect). Amongst these factors and interactions, only the factor alcohol-water ratio had a significant effect on the EA as shown by the variance analysis. The other factors and interactions had a slight influence on the EA. The validation elements for the model are contained in Table 10 as follows:

Table 10. Validation of the EA model for pectin of C. M.

Validation elements	Abbreviations	Observed values	Standard values
Coefficient of determination	R^2	82.7901%	100%
Absolute mean deviation analyses	AMDA	0.14789617	0

The R^2 was 82.7901 and the AMDA which was very small and approximately zero permitted the validation of the model. The surface response of the EA of the pectin of C. M. was plotted as a function of the pH, alcohol/water ratio, with the temperature being maintained fixed within the centre points of the experimental domain Figs. 8 and 9.

The EA attained an optimum at pH equal to or higher than 3 for an alcohol-water ratio equal to or higher than 2.5, and a temperature range between 70 and 90°C. At pH equal or higher than 3, there is an attenuation of the effect of severe temperatures combined with the extreme acidic pH. These conditions thus make it possible for pectin of C. M. to preserve their structural integrity. This maintains the viscosity of the macromolecule which slows molecular motion and hence facilitates the formation of emulsions.

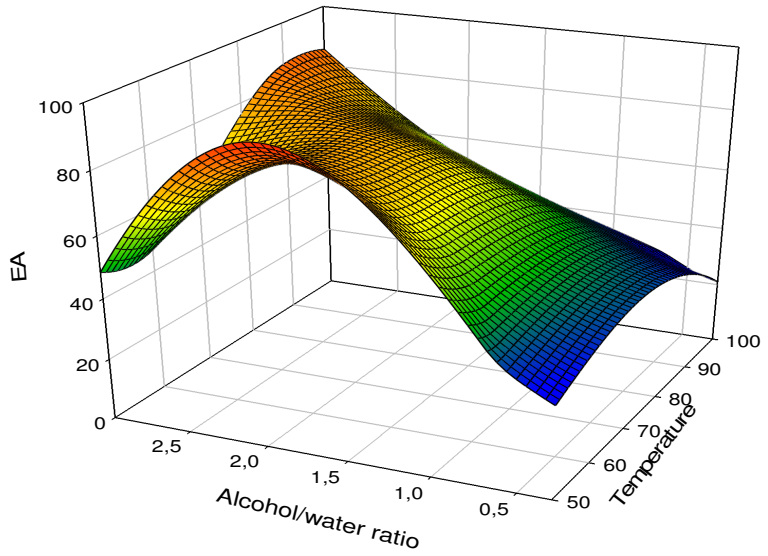

Fig. 8. Evaluation of the AE of pectin of C. M. as a function of the alcohol/water ratio and the temperature EA: : 0% ; : 20% ; : 40% ; : 60% ; : 80% ; : 100%; :120%

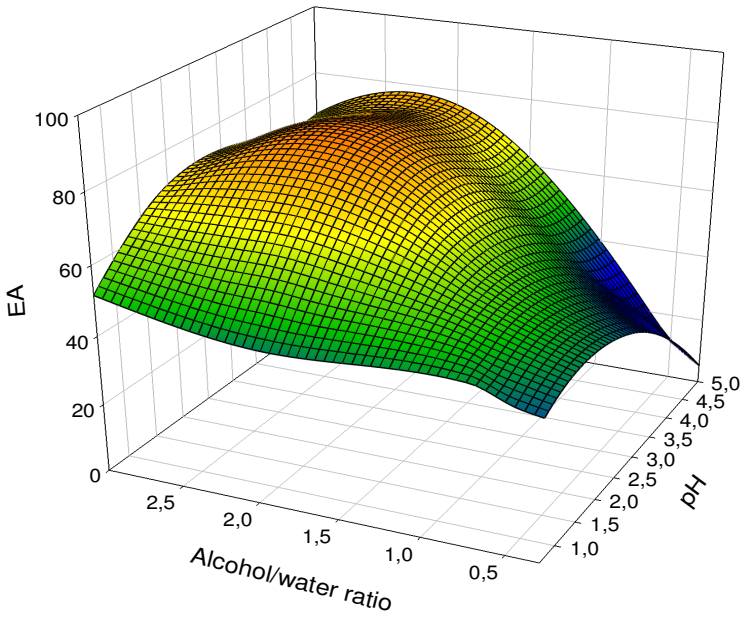

Fig. 9. Evaluation of the EA of the pectin of C.M. as a function of the alcohol/water ratio and the pH EA: : 0% ; : 20% ; : 40% ; : 60% ; : 80% ; : 100%; :120%

4. DETERMINATION OF THE OPTIMUM POINTS

With the application of response surface methodology 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 difficult 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 enables us to determine the optimum points for analysed responses.

Table 11. Determination of the optimum zone of the pectin of C. M.

Factors	Yields	CAEa	SI	EA	Optimum
pH	1.5	2–3.5 4–4.5	>4	≥3	2.4
Alcohol-water ratio	1	1.5–2.5 >3	1.5–2.5	≥2.5	1.5
Temperature (ºC)	60	70-90	70-80	70-90	74

From this table, the obtained P.C.M. is optimised at a pH of 2.4, a ratio of 1.5 and a temperature of 74°C.

5. CONCLUSION

The optimisation of the extraction of pectin from CM was the main focus in this work. The main results show that, the only factor which had a significant effect on the extraction of pectin from C.M. was pH, whereas temperature influenced the process slightly. Extraction of pectin from C.M. was achieved at optimum conditions of pH 2.4, an alcohol-water ratio of 1.5

and a temperature of 74°C. The optimisation of the extraction of pectin considered was thus carried out. However the study of the effect of other factors could contribute to improve the optimisation of the extraction yield.

ACKNOWLEDGEMENTS

This research work was supported by "Agence Inter- établissements de Recherche pourle Développement" (AIRD).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ndjouenkeu R. Functional properties of some African food hydrocolloids. Ph.D dissertation, University of Ibadan, Nigeria. 1995;57-287.
2. Ndjounkeu R, Goycoolea FM, Morris ER, Akingbala JO. Rheology of okra (*Hibiscus esculentus* L) and dika nut (*Irvingia gabonensis*) polysaccharides. Carbohydrate Polymers. 1996;29:263-269.
3. Ptichkina NM, Danilova IA, Doxastakis G, Kasapis S, Morris ER. Pumpkin pectin: Gel formation at unusually low concentration. Cabohydrate Polymers. 1994;23:265-273.
4. Ralet MC, Bonin E, Thibault JF. Pectins, dans: Biopolymers Polysaccharides II, Steinbuchel A. (Ed.), Wiley-VCH Verlag Gmbh, Weinheim. (2002;8(12):345-380.
5. Kouebou C, Achu , Nzali S, Chelea M, Bonglaisin, Kamda A, Djiele P, Yadang RG, Ponda R, NgohNewilah G, Nkouam G, Teugwa C. Kana Sop. A review of composition studies of Cameroon traditional dishes: Macronutrients and minerals. Food Chemistry; 2013.
6. Saidou. Propriétés physic-chimiques et fonctionnelle des gommes hydrocolloides des écorses de *Triumfetta cordiofilia* (*Tiliacée*) et de Bridelia thermifolia (Euphorbiacée). Université de Grénoble, France; 2012.
7. Anderson R, Conway H, Pfeifer V, Griffin E. Roll and extrusion-cooking of grain sorghum grits. Cereal Science Today. 1969;14:372-375.
8. Ptichkina NM, Ishin AG, Danilova IA. Method of pectin production. Obvious USSR 5035191/13; 1996.
9. Ralet MC, Bonin E, Thibault JF. Pectins, dans: Biopolymers vol. 8, Polysaccharides II, Steinbuchel A. (Ed.), Wiley-VCH Verlag Gmbh, Weinheim. 2002;12:345-380.
10. Phillips RD, Chinnan MS, Branch AL, Miller J, Mc Watters KH. Effects of pretreatment on functional and nutritional properties of cowpea meal. J. Food Sci. 1988;53(3):805-809.
11. Muschiolok G. Behaviour of low-calory spreads based on protein (stabilised oil-in-water systems. In: Dickinson E.(ed.): Fod polymers, gels and colloids. The Royal Societyof Chemistry, Cambridge. 1991;232-243.

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