Characterization, optimization and classification of LM and HM-pectins from mango peel, passion fruit rinds, and Bambara groundnut shells wastes mixtures

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Abstract- *This study aims to characterize, to optimize, and to classify the pectins obtained from the mixtures of mango peel, passion fruit rinds, and Bambara groundnut shells wastes. A Mixture Experiments with Process Variables (MPV) design was applied. The low level of the process variables (pH and liquid/solid ratio: LSR) were set at 1.5 and 12.5ml/g respectively, the high level at 2 and 15ml/g. Citric acid was used as extracting agent in two-stepped extraction at 80°C and 60 minutes separately. The yield, equivalent weight (EW), methoxyl content (MeO), anhydrouronic acid content (AUA), and the degree of esterification (DE) of the 28 experimental points were determined. The yields and the qualities of the extracted pectins depended on the plant sources, the mix ratio, the pH and the LSR. Mango and passion fruit wastes gave good yields of pectins and high AUA contents. In contrast, Bambara groundnut waste provided moderate yield and appreciable values of EW, MeO and DE. This complementarity created the opportunity to achieve final products that can compete commercial pectins by mixing the plant sources, particularly with Mango-Bambara groundnut (MB) mix. The use of MPV design offered the possibility of scanning pectins that meet the standard specifications at the experimental domain. Good qualities and high-yielded LM-pectin (LMP), and HM-pectins (HMP) can be extracted at the same time. The optimum yields were 40.3% for LMP (MB: 31.3% - 68.7%), 32.0% for slow set pectin (MB: 56.6% - 43.4%), 27.5% for medium set pectin (MB: 95.0% - 5.0%), and 19.0% for rapid set pectin (MB: 54.6% - 45.4%). The presence of Bambara groundnut in the plant wastes mixtures is the key factor of the procurement of marketable pectins.*

Keywords- *pectin, wastes mixtures, MPV design, characterization, optimization, classification*

1. INTRODUCTION

Pectin is a family of complex polysaccharides present within the primary cell wall and intercellular areas of dicotyledons. Pectins are methylated ester of polygalacturonic acid that contains 1,4-linked-D-galacturonic acid residues [1]. This structure involves 300-1000 chains of galacturonic acid units [2]. High methoxyl pectins (HMP) have more than half the carboxyl groups as methyl esters and form gels in the presence of high sugar concentrations and acid. Low methoxyl pectins (LMP) have less than half the carboxyl groups as methyl esters [3], they form gels in presence of cations as calcium ions [4].

Most commercial pectins, commonly intended for the preparation of gelling food products, are produced from citrus (lemon, lime, orange, and grapefruit) peel and apple pomace [5-8]. On a dry weight basis, the two industrial by-products are pectin-rich sources, with a pectin content of 15–30 % (for citrus peel) and 10–15 % (for apple pomace). But, there are other commercially viable sources of pectins: sunflower head residues (for naturally LMP), sugar beet pulp (for manufacturing pectin emulsifiers), and some tropical fruit by-products such as mango peel (for HMP), yellow passion fruit rind (for naturally HMP and LMP), and cashew apple pomace (for naturally LMP) [9-10]. Passion fruit rind, for example, is used in some emerging countries such as Brazil for manufacturing marketable pectins [11].

Besides, our recent research stipulated that the mixing of the plant wastes from mango peel (*Mangifera indica*) recovered from *fody* variety, yellow variety of passion fruit rinds (*Passiflora edulis*) and creamy brown-striped Bambara groundnut shells (*Vigna subterranea*) had a significant synergetic effect on the mixes' pectin yields permitting to the procurement of high yielded-pectins [12]. However, these achieved pectins need to be characterized, the yields and the qualities of these pectins must be optimized in order to find marketable pectins fitting the standard specifications. The functional properties of pectins as well as equivalent weight (EW), methoxyl content (MeO), anhydrouronic acid (AUA) content and the degree of esterification (DE), are highly correlated to their structures, which depend on the plant sources and the extraction method used, establishing variations in the content and quality [13].

The Minitab® 18.1 Software (Minitab Inc., State College, PA, USA) provides the Mixture Experiments with Process Variables (MPV) design which allowed to fulfill the aim of this research [14-15]. The polynomial models of the aforementioned physic-

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chemical parameters will be applied to predict at once the optimum yields and the optimum qualities of different categories of pectins that can be obtained from the experimental design.

2. MATERIALS AND METHODS

2.1. Preparation of the plant sources

The mango peel from *fody* variety, the passion fruit rinds from yellow variety, and the shells of creamy brown-striped Bambara groundnut were recuperated from the markets of Fianarantsoa, Madagascar. Immediately afterward, these wastes were treated at the LPS laboratory. After washing, sorting and weighing, they were dried in an oven at 50°C until constant weight. The dry wastes were then powdered with a pestle and mortar and passed through a 0.6mm diameter sieve. The powders were weighed and stored individually in plastic vessels.

They were pretreated twice with 85° ethanol at 70°C for 20 minutes under reflux to remove soluble ethanol impurities (sugars, pigments, etc.) [16]. The insoluble fractions were dried in an oven at 50°C and then stored in the freezer at -18°C before further use.

2.2. Experiment designs

The Mixture Experiments with Process Variables (MPV) design [14-15], provided by Minitab® 18.1 Software (Minitab Inc., State College, PA, USA) was uses. It is an association of the simplex-centroid mixture design [17] of the 3 components (mango, passion fruit, and Bambara groundnut) illustrated in figure 1 and the full factorial design of the process variables (pH and LSR) in table 1.

The full factorial design is the association of the low level (-) and the high level (+) of the process variables. It contains 4 runs. The pH levels were fixed at 1.5 and 2 but the Liquid/Solid ratio (LSR) were set at 12.5ml/g (150ml/12g) and 15ml/g (150ml/10g). Thus, the MPV design has 28 runs (Table 2). The extraction yields, the EW, the MeO, the AUA content and the DE were evaluated for each experimental point. The experiments were repeated twice.

2.3. Pectin extraction

Pectins were extracted using citric acid and the technique of sequential two-step extraction [18]. The temperature and the extraction time were 80°C and 60 minutes respectively [19]. After 60 minutes, the mixtures were sieved through a polyester cloth and then cooled to room temperature. The residues were subject to a second extraction under the same conditions. The second filtrates were poured to the first ones and stored in a refrigerator at 4°C. Double volumes of 96° ethanol were added to the refrigerated filtrates. They were kept for 1 hour to precipitate the pectins. The precipitates were washed twice with 70° ethanol then once with 96° ethanol. The purified pectins were stored at 4°C overnight and then dried in a 50° oven until constant weight.

2.4. Pectin yields

Pectin yields were calculated using equation 1.

$$Y_{pec\,(\%)} = \left(\frac{P}{P_0}\right) \times 100 \qquad (1)$$

Where *Ypec* (%) is the pectin yield, *P* is the weight of the pectin after drying. P_0 is the initial weight of waste powder taken individually or blended before pre-treatment with 80° ethanol at 70°C during 20 minutes.

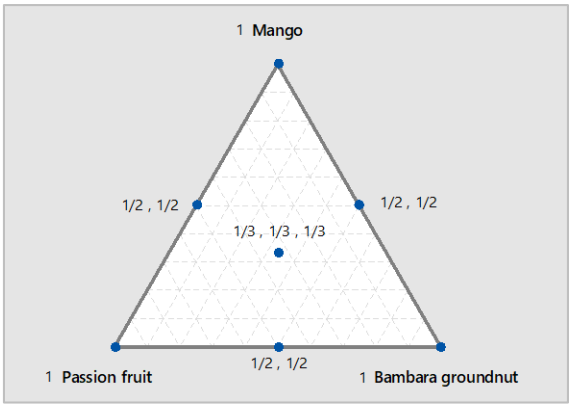

Figure 1: Simplex-centroid design of the three plants sources

Table 1: Full factorial design of the process variables

Process variables 4 runs		
Run	pH	LSR
1	-	-
2	+	-
3	-	+
4	+	+

Table 2: MPV design of the whole experiments

	Process variables (pH*LSR)			
Type of Mixture	1	2	3	4
M 100%	- -	+ -	- +	+ +
P 100%	- -	+ -	- +	+ +
B 100%	- -	+ -	- +	+ +
MP 50% - 50%	- -	+ -	- +	+ +
MB 50% - 50%	- -	+ -	- +	+ +
PB 50% - 50%	- -	+ -	- +	+ +
MPB 33.3% - 33.3% - 33.3%	- -	+ -	- +	+ +

M: Mango, P: passion fruit, B: Bambara groundnut

2.5. Equivalent weight (EW)

Equivalent weight is used to calculate the anhydrouronic acid (AUA) content and the degree of esterification (DE) according to Owens *et al*. method (1952) [20]. It is assessed by titration with sodium hydroxide to pH 7.5 using either phenol indicator. 0.5g of pectin substances (ammonia and ash-free) was weighed into a 250-ml conical flask. Then, it was diluted with 5ml ethanol. 1g of sodium chloride was added to sharpen the end point. 100ml of distilled water was added together with six drops of phenol red or Hinton's indicator. All the pectin substances are made sure to have dissolved. Then, the solution was titrated slowly with 0.1N NaOH.

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Titration point was indicated by purple color. It can be determined by the equation below.

$$Equivalent\ weight\ = \frac{Weight\ of\ sample\ \times 1000}{ml\ of\ alkali\ \times Normality\ of\ alkaly} \quad (2)$$

2.6. Methoxyl Content (MeO)

The methoxyl content is an important factor in controlling the setting time of pectins, the sensitivity to polyvalent cations, and their usefulness in the preparation of low solid gels, fibers and film. It is measured by saponification of the pectin and titration of the liberated carboxyl groups. Determination of MeO was done by using the Ranganna's method (1986) [21]. The neutral solution was collected from determination of EW, and 25 ml of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 ml of 0.25 N hydrochloric acid was added and titrated against 0.1 N NaOH to the same end point as before like in equivalent weight titration [22].

$$Methoxyl\ content\ \% \ = \frac{ml\ of\ alkali \times Normality\ of\ alkali \times 31 \times 100}{Weight\ of\ sample \times 1000} \quad (3)$$

Where 31 is the molecular weight of methoxyl.

2.7. Total Anhydrouronic Acid Content (AUA)

Estimation of AUA content is essential to determine the purity and degree of esterification, and to evaluate the physical properties. Pectin, which is a partly esterified polygalacturonide, contains 10% or more of organic material composed of arabinose, galactose and perhaps sugars. Making used of the equivalent weight and methoxyl content value of titre used. Total AUA of pectin was obtained by the following formula [23].

$$\%\ of\ AUA\ = \frac{176\ \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000} \quad (4)$$

Where molecular unit of AUA (1 unit) = 176 g
Where, z = ml (titre) of NaOH from equivalent weight determination.
y = ml (titre) of NaOH from methoxyl content determination.
W = weight of sample

2.8. Determination of Degree of Esterification (DE)

The DE of pectin was measured on the basis methoxyl and AUA content [24] and calculated by flowing formula.

$$\%\ DE = \frac{175 \times \%\ MeO}{31 \times \%\ AUA} \times 100 \quad (5)$$

2.9. Regression models

The regression models of the responses (yields, EW, MeO, AUA content, and DE) were calculated by the mean of the MPV design provided by the Minitab® 18.1 Software (Minitab Inc., State College, PA, USA). Equation 9 gives the regression model which results from the crossing (Equation 8) of the Scheffé's quadratic model for components in Equation 6, and the full factorial model for the process variables in Equation 7. The full model contains 24 terms [12, 14].

$$f(x) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (6)$$

$$g(z) = \alpha_0 + \alpha_1 z_1 + \alpha_2 z_2 + \alpha_{12} z_1 z_2 \quad (7)$$

$$R_{x,z} = f(x) \times g(z) \quad (8)$$

$$R_{x,z} = \gamma_1^0 x_1 + \gamma_2^0 x_2 + \gamma_3^0 x_3 + \gamma_{12}^0 x_1 x_2 + \gamma_{13}^0 x_1 x_3 + \gamma_{23}^0 x_2 x_3 + \gamma_1^1 x_1 z_1 + \gamma_2^1 x_2 z_1 + \gamma_3^1 x_3 z_1 + \gamma_1^2 x_1 z_2 + \gamma_2^2 x_2 z_2 + \gamma_3^2 x_3 z_2 + \gamma_{12}^1 x_1 x_2 z_1 + \gamma_{13}^1 x_1 x_3 z_1 + \gamma_{23}^1 x_2 x_3 z_1 + \gamma_{12}^2 x_1 x_2 z_2 + \gamma_{13}^2 x_1 x_3 z_2 + \gamma_{23}^2 x_2 x_3 z_2 + \gamma_1^{12} x_1 z_1 z_2 + \gamma_2^{12} x_2 z_1 z_2 + \gamma_3^{12} x_3 z_1 z_2 + \gamma_{12}^{12} x_1 x_2 z_1 z_2 + \gamma_{13}^{12} x_1 x_3 z_1 z_2 + \gamma_{23}^{12} x_2 x_3 z_1 z_2 \quad (9)$$

$R_{x,y}$ represents the overall values of the responses. x_i's are the linear terms of components, $x_i x_j$'s are the quadratic terms of components. $x_i z_l$'s are the interaction terms of the individual sources with the pH or the LSR. $x_i x_j z_l$'s are the interaction terms of the mixes with the pH or the LSR. $x_i z_l z_m$'s are the interaction terms of the individual sources with the pH and the LSR, and $x_i x_j z_l z_m$'s are the interaction terms of the mixes with the pH and the LSR. The γ's are the coefficients of the model. The lower indexes of γ refer to the components, whereas the upper ones refer to process variables.

2.10. Data processing

Data were treated using the Minitab® 18.1 Software (Minitab Inc., State College, PA, USA). The model accuracy was tested by R^2, adjusted R^2, predicted R^2 and value of S or the "standard error of the model" [24]. A lower value of S indicates a better fitting models. The values of S, R^2 and adjusted R^2 indicate how well the models fit the observed data. The value of predicted R^2 is indicator of how well the regression models predict new observations [25]. The regression models were validated by the ANOVA of the regression terms (p-value<0.05).

The effect of the mixing and the process variables on the quality of the pectins were evaluated by the mean of the mixture contour plots of the EW, the MeO, the AUA contents and the DE.

The preponderant regions corresponding to the optimum yields and the optimum qualities of pectins were scanned with the overlaid contour plot provided by the Minitab Software. It identifies regions on a contour plot where the fitted models predict acceptable results for both the quantitative and the qualitative responses. The optimal points at these regions were identified by the mean of the response optimizer tool accessible in the Minitab Software. It identifies the combination of predictor settings that jointly optimize the fitted responses.

The field of the reasearch was divided into 4 parts according to the value of the DE: between 0 and 50 for LMP, between 58 and

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65 for slow set HMP, between 66 and 69 for medium set HMP, and between 71 and 74 for rapid set HMP [26]. The limits of the other responses were fixed with agreement to the standard specifications and according to their minimum or maximum limits. Theses limits were: between 6% and 48% for the extraction yields, between 600 [27] and 1158 for the EW, between 0.5 and 7 for the MeO of LM-pectins, between 7 and 12 for the MeO of HM-pectins, and between 65% [28] and 88% for AUA content.

3. RESULTS

3.1 Quantitative and qualitative responses

The averages of the quantitative (yield) and the qualitative responses (EW, AUA content, DE, and MeO) from the 28 experiments with 2 replicates are given in table 3.

3.2. Validity of the models

Table 4 shows the Model coefficients of the extraction yield, the EW, the AUA content, the MeO, and the DE. The high F-values (3297.59, 2309.13, 239.37, 335.11, and 576.39 respectively) and the p-values (< 0.0001) demonstrated the validity of the models. The coefficients between brackets are not significant according to the ANOVA tests. The values of S (0.32, 5.6, 0.9, 0.18, 0.84 respectively), the values of R^2 (0.9996, 0.9994, 0.9942, 09959, and 0.9976 respectively) and the values of adjusted R^2 (0.9993, 0.9990, 09901, 0.9929, and 0.9959 respectively) of the responses indicate that the models fit the observed data. The values of the predicted R^2 (0.9987, 0.9980, 0.9789, 09.845, and 0.9911 respectively) and the irrelevant p-values of lack of fit performance (0.737, 0.104, 0.112, 0.548, and 0.371 respectively) indicate that the predicted values could reasonably represent the experimental values of all the responses [25, 29]. The predicted models of the yields, EW, AUA contents, MeO, and DE could be utilized to the optimization of the responses at the experimental domain.

3.3. Characterization of the assessed pectins

The EW, AUA content, MeO and DE of pectins from individual sources and from the mixtures in table 3 were analyzed to characterize them. The effect of the mixing and the process variables (pH and LSR) were appreciated by the mean of the mixture contour plot. The linear and quadratic terms in table 4 were utilized to explain the contour plots.

3.3.1. Mixture contour plot of the EW

The EW of the samples ranged from 350 to 1054. The linear terms of EWs (Table 4) of mango and passion fruits (527.66 and 508.44 respectively) were under the standard specification (> 600) [27]. It was not the case of Bambara groundnut (797.34). Low pH tend to decrease the EWs. At pH 1.5, the EWs of mango and passion fruits-rich mixtures were all under 400 but the EWs of Bambara groudnut rich mixtures remained up to 600. The values of EWs at pH 2 were all superior to 600. The EWs of Bambara grounut-rich mixture reach up to 900 at this condition. The high LSR improved slighty the EWs except for Bambara groundnut (figure 2).

3.3.2. Mixture contour plot of the AUA contents

The means of AUA contents (Table 4) of mango and passion fruits (82.30% and 82.23% respectively) fit the standard specification (>65%). The case of Bambara groundnut was different (63.55%). Nevertheless, its AUA contents could meet this standard at high pH. The AUA contents of the individual sources and the mixes increased with the augmentation of pH from 1.5 to 2.

Tableau 3: Experiment results

	MPV design			Responses				
Run	Mix	pH	LSR	Yield	EW	AUA	MeO	DE
1	M	1.5	15	42.9	368.9	82.2	6.1	42.0
2	P	1.5	15	34.2	392.3	85.3	7.1	47.4
3	B	1.5	15	23.9	694.4	47.0	3.8	46.1
4	MP	1.5	15	40.8	387.8	77.5	5.6	41.4
5	MB	1.5	15	45.5	540.8	66.2	5.9	50.8
6	PB	1.5	15	31.4	502.8	69.0	6.0	49.2
7	MPB	1.5	15	41.1	473.5	69.4	5.7	46.3
8	M	1.5	12.5	43.8	352.0	84.0	4.7	31.4
9	P	1.5	12.5	24.4	386.4	82.0	6.4	44.3
10	B	1.5	12.5	24.1	591.0	62.8	5.4	48.7
11	MP	1.5	12.5	31.3	336.0	84.0	5.4	36.5
12	MB	1.5	12.5	39.1	447.2	74.1	6.1	46.6
13	PB	1.5	12.5	21.6	428.4	73.5	5.7	43.8
14	MPB	1.5	12.5	30.9	386.4	78.4	5.8	42.1
15	M	2	15	29.2	743.5	76.4	9.3	69.0
16	P	2	15	25.1	631.7	85.9	10.2	67.6
17	B	2	15	13.8	861.3	75.0	9.6	72.7
18	MP	2	15	28.0	624.2	84.5	9.9	66.6
19	MB	2	15	18.4	608.6	82.4	9.4	64.9
20	PB	2	15	21.6	768.6	63.4	7.1	63.9
21	MPB	2	15	22.5	628.1	77.3	8.7	63.7
22	M	2	12.5	10.4	645.2	86.6	10.4	68.5
25	P	2	12.5	9.6	622.3	75.7	8.3	62.6
24	B	2	12.5	5.8	1041.5	69.4	9.5	77.8
25	MP	2	12.5	12.8	588.2	78.8	8.6	62.1
26	MB	2	12.5	11.5	836.1	84.2	11.1	75.0
27	PB	2	12.5	9.0	693.7	85.2	10.5	70.0
28	MPB	2	12.5	12.1	687.0	83.8	10.2	69.4

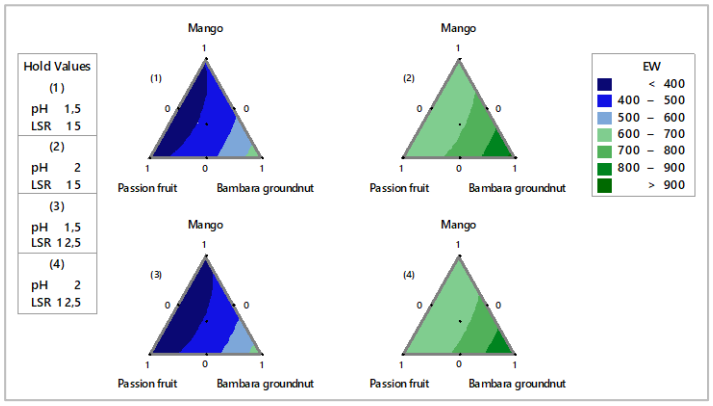

Figure 2: Multiple mixture contour plot for EW

Low LSR also affected positively the AUA contents. High pH and low LSR medium was the best condition to conserve the maximum of the AUA contents (figure 3).

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3.3.3. *Mixture contour plot of the MeO*

The MeO contents can be clasified into two categories: between 0.5% and 7% for LMP, and between 7% and 12% for HMP [26]. Extraction at pH 1.5 gave a value of MeO under 7%. But the values of the MeO at pH 2 ranged between 9% and 11% (figure 4).

3.3.4. *Mixture contour plot of the DE*

When the pH was set at 1.5, the DEs were under 50% except for Bambara groundnut which DEs ranged between 50 and 55%. At pH 2, the DEs were above 50% and can reached up to 70% for Bambara groundnut. High LSR increased the DEs of mango and/or passion fruit-rich mixtures but it decreased the DEs of Bambara groundnut-rich pectins (figure 5).

Table 4: Model coefficients of the responses

	Terms	Yield	EW	AUA	MeO	DE
Linear terms	M	31,569	527.66	82.30	7.62	52.75
	P	23,313	508.44	82.23	8.03	55.51
	B	16,863	797.34	63.55	7.08	61.36
Quadratic terms	MP	3,273	-140.38	-4.25	-1.76	-10.28
	MB	17,687	-221.52	15.21	3.10	8.73
	PB	3,285	-222.42	(-0.46)	-0.97	-7.10
Interaction terms with pH	M*pH	-11,783	167.05	-0.80	2.25	15.98
	P*pH	-5,966	118.93	-1.46	1.25	9.56
	B*pH	-7,1	154.44	8.62	2.47	13.89
	M*LSR	4,464	29.00	-2.97	(0.07)	2.79
	P*LSR	6,328	4.05	3.38	0.65	2.04
	B*LSR	1,955	-18.98	-2.57	-0.37	-1.91
Interaction terms with LSR	MP*pH	4,154	-84.72	6.70	(0.59)	(0.45)
	MB*pH	-16,994	-187.77	10.91	-0.81	-16.49
	PB*pH	3,696	(-17.00)	-8.04	-1.38	-5.32
	MP*LSR	3.046	18.49	(-1.68)	(0.10)	(-0.57)
	MB*LSR	(0.295)	-157.10	(1.30)	-1.28	-8.02
	PB*LSR	5.658	176.01	-28.04	-3.65	-1.41
Interaction terms with pH and LSR	M*pH*LSR	4.916	20.68	-2.18	-0.65	-2.51
	P*pH*LSR	1.396	(1.18)	1.65	0.28	(0.44)
	B*pH*LSR	2.057	-70.59	5.28	0.41	(-0.61)
	MP*pH*LSR	-6.966	-64.55	14.38	1.92	3.97
	MB*pH*LSR	-13.554	-226.30	(1.04)	-0.94	-7.97
	PB*pH*LSR	-4.144	134.29	-29.94	-4.94	-11.11
R^2		0,9996	0.9994	0.9942	0.9959	0.9976
R^2-adjusted		0,9993	0.9990	0.9901	0.9929	0.9959
R^2-predicted		0,9987	0.9980	0.9798	0.9845	0.9911
F-value of the model		3297.59	2309.13	239.37	335.11	576.39
p-value of the model		<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Lack of fit		0.737	0.104	0.112	0.548	0.371
S		0,32	5,6	0,9	0,18	0,84

3.4. Optimization

The preponderant regions of optimum yields of marketable LMP and HMP were first scanned using Minitab® 18.1 Software (Minitab Inc, State College, PA, USA) by varying the pH and the LSR between their low and their high levels. The seeking of the preponderant regions allowed to go more quickly with the optimization. Then, the yield, the EW, and the AUA content were optimized at once. The DE and the MeO served for the classification of the pectins as LMP and HMP (slow set. medium set. and rapid set).

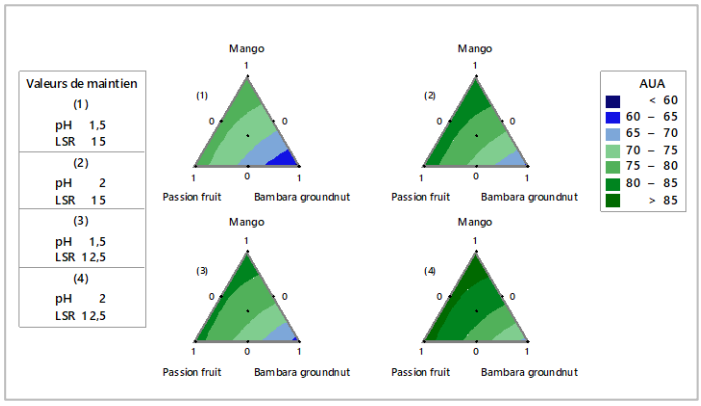

Figure 3: Multiple mixture contour plot of AUA contents

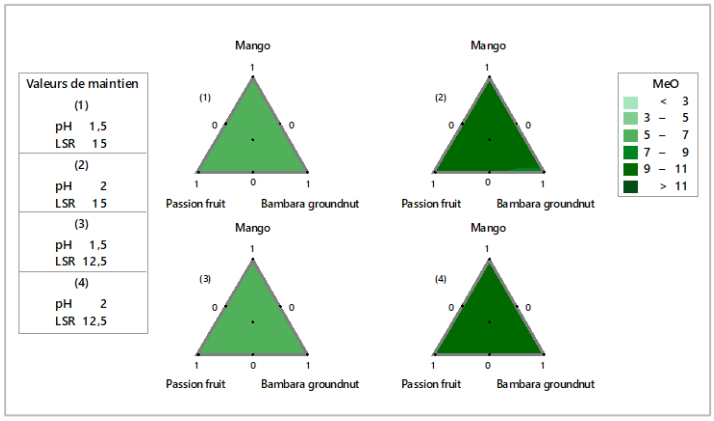

Figure 4: Multiple mixture contour plot of the MeO

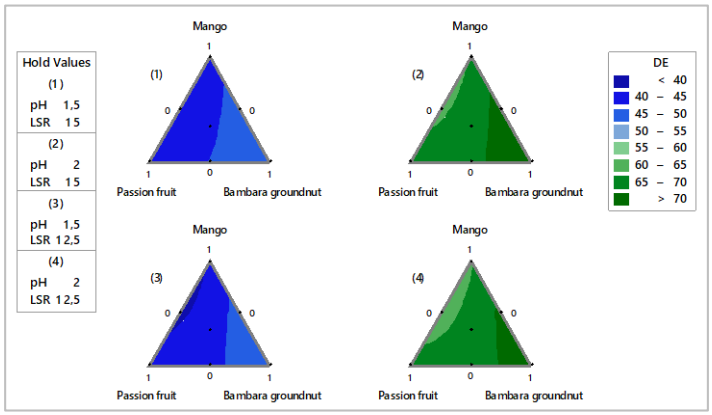

Figure 5: Multiple mixture contour plot of the DE

3.4.1. *Preponderant region for LMP*

The preponderant region for LMP in the mixture design was predicted by Minitab® 18.1 Software using the responses' models (table 4). The software utilized the predicted model of each response and drew their contour plots in the mixture design. The white-colored area in figure 6 corresponds to the preponderant

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region of LMP. Production of marketable LMP is possible at the region of MB mix where Bambara groundnut is present at high proportion.

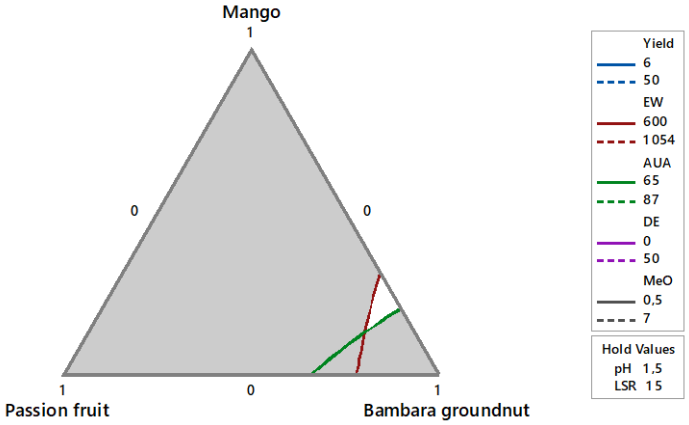

Figure 6: Preponderant region for LMP

3.4.2. Preponderant region for HMP

Five preponderant regions for the HMP (white area) were scanned at the mixture design by varying the pH and the LSR and by defining the suitable limits of the important parameters as yield, EW, MeO, AUA contents and DE. The group of three regions, observable at pH 1.75, belongs to the area of the mixes with high proportion of Bambara groundnut. The second group of preponderant regions is detected at pH 2. It widened with the growth of the LSR, extending from the area of MP mix until the area of MB mix (Figure 7).

3.4.3. classification of the optimized pectins

The three responses (yield, EW, AUA contents) were optimized together by fixing the suitable values of the DE and the MeO corresponding to the category of the desired pectin.

Table 5 shows the optimized pectins in term of yields, EW and AUA contents. They were also classified into four categories according to the values of the MeO and the DEs. The optimal conditions of the procurement of LMP is at pH 1.5 and LSR 15ml/g. The optimal yield reached up to 40.6%. This category of pectin derived from MB mix where the proportion of mango is 31.3%, the percentage of Bambara groundnut attains 68.7%. Its EW (600.2) and AUA content (67.9%) fit the standard specification.

Three categories of optimized HMP can be scanned from the preponderant regions. Good yield of slow set pectin (32.0%) will be available at pH 1.77 and LSR 15 ml/g from the proportion of 56.6% of mango peel and 43.4% of Bambara groundnut shell. The EW and the AUA content of this pectin are respectively equal to 606.4 and 75.9%. An optimum yield of 27.5% of medium set pectin will be assessed at pH 2 and LSR 14.96 ml/g with the mix composed by 95% of mango and 5% of Bambara groundnut. The EW reaches up to 669, the AUA content is excellent (82.3%). Finally, 19% of rapid set pectin will be extracted from the mix composed by 54.6% of mango and 45.4% of Bambara groundnut at pH 2 and LSR 15 ml/g. The EW is better than the previous types (733.8), the AUA content is equal to 78%.

Table 5: Optimization and classification of pectins

Type of pectin	Proportion MB (%)	pH	LSR	Yield (%)	EW	AUA (%)	MeO	DE
LMP	31.3/68.7	1.5	15	40.3	600.2	67.9	5.88	49.0
Slow set	56.6/43.4	1.77	15	32.0	606.4	75.9	8.15	59.9
Medium set	95.0/5.0	2	14.96	27.5	669.0	82.6	9.52	66.1
Rapid set	54.6/45.4	2	15	19.0	733.8	78.0	9.92	71.3

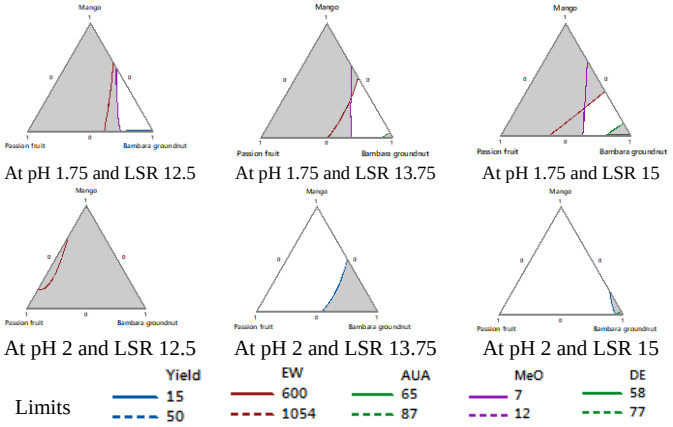

Figure 7: Preponderant regions for HMP

4. DISCUSSIONS

4.1. Characterization

4.1.1. Equivalent weight (EW)

The EW is the amount in grams of pure polygalacturonic acid. It depends on the degree of esterification, which is correlated with the number of free carboxylic groups in one gram mole equivalent to one gram mole of hydroxy [21]. The EW of pectin samples indicates its jelly-forming ability, wherein better gelling agents possess higher equivalent weight [30].The EW of all mixtures ranged from 350 to 1054, it was somewhat under the EW of lemon pomace pectin comprised from 368-1632, publised by Azad et al. (2014) [31]. Nahar *et al.* (2017) [32] found that EW of mango peel cultivars varied from 506.58-2142 depending on the extraction condition used. They found a value of 654.14±0.67 using citric acid at pH 1.5 and LSR 20ml/g. At the same pH and at LSR15ml/g, the EWs of mango, passion fruit and bambara groundnut were respectively: 368.87, 390.6 or 392.32 and 694.44. This comparison confirms that high LSR improves the EW of mango.

The values of the EW of pectins using citric acid were often under those extracted by other types of acid. Uzma et al. (2015) [33], stipulated that EW of papaya peel pectin extracted using HCl and citric acid was 912.17 and 455.1 respectively. Devi *et al.* (2014) [34], demonstrated that the EW of pectin extracted from sweet lemon peel powder using citric and nitric was found to be 312.5 and 833.33 respectively.

The standard of EW published by International Pectin Producers Association (IPPA) in 2002 [27] ranges from 600-800 mg. At pH 1.5, most of the EW of components were inferior to this standard except for Bambara groundnut. For individual sources, the EWs

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were decreased significantly with the lessening of the pH. Nazaruddin (2011) [35] also argued that the decreasing of the EW depends upon the amount of free acid. However, mixing with bambara groundnut improves the EW of the samples even at low pH. This is the reason why the optimized LMP meets the standard specification with the mix composed by 31.3% of mango and 68.7% of Bambara groundnut (table 5).

4.1.2. Anhydrouronic acid content (AUA)

The AUA content indicates the purity of pectin. The value of AUA content should not be less than 65% [27, 36]. AUA content of less than 65% may indicate impurities due to the presence of proteins, starch, ash, and sugars in the precipitated pectin [37]. So, pectins from mango and passion fruit (average of AUA contents 82.3% and 82.2% respectively) are purer than pectin from Bambara groundnut (mean of AUA contents 63.5%). The low purity of Bambara groundnut shell pectin may be proven by its high ash and its high protein contents. To confirm this, Mahala and Mohammed (2010) [38] published the chemical composition of Bambara groundnut shell containing 15.306% of crude protein, 25.193% of crude fiber, 3.523% of fat and 3.845% of ash. The overall yield of Bambara shell pectin found in this research (16,863%) is in accordance with this percentage of crude fiber.

The best condition to the procurement of good amount of AUA was at pH 2 and LSR 12.5 ml/g for all samples. Thomas *et al*. (2008) affirmed that lower pH values negatively affected the galacturonic acid content of pectin, but increased the pectin yield. It seems that, for some species like Bambara groundnut, the low pH induces the mobilization of the other components (divalent cations, starch, and proteins) that contaminates the pectin. Several authors published low AUA content at pH 1.5: (*i*) 57.32% for ripe banana peel and 39.68% for unripe banana peel using HCl [39], (*ii*) 65.4% for lemon peel using nitric acid [40], (*iii*) 63.2% with honeydew (*Cucumis melo l. var. inodorous*) using citric acid [41].

The increasing of the LSR lessened the amount of AUA. The water activity would provoke the mobilization of protein and polyphenols [42] which reduce the purity of the pectins. Mixing with Bambara groundnut decreased the purity of the mix. However, the purity of the three categories of the optimized Bambara groundnut mixes found in this research satisfies the standard specification: 75.9% for slow set MB mix pectin, 82.6% for medium set MB mix pectin, and 78.0% for rapid set MB mix pectin (table 5).

4.1.3. Methoxyl content (MeO)

The MeO content is an important factor in controlling the setting time of pectins and the ability of pectin to form gels [43]. Pectin's spreading quality and sugar binding capacity were increased with increasing MeO content [44]. The methoxyl content also is an important variable that determines the sensitivity of its response to polyvalent cations. If the methoxyl content is high, it indicates that the pectin will gel quickly. Regular pectins have a methoxyl content between 7% and 12% and are also called high-methoxyl pectins (HMP). Pectins with a methoxyl content between 0.5% and 7% are known as low-methoxyl pectins (LMP). LMP are used in the manufacture of dietetic and lower-calorie fruit jellies [26].

At pH 1.5, the MeO of all the components was under 7%. That is the reason of the possibility of obtaining LMP from mixtures at this condition. At pH 2, the values of the MeO were superior to 7%. At this condition, all the components are able to form gels in presence of sugar. This is the reason why three types of HMP are available at this condition.

4.1.4. Degrees of Esterification (DE)

DE is the identification parameter for classifying of pectins. DE > 50% are known as HMP and a DE < 50% are LMP [45]. HMP forms gels under acidic conditions (pH < 4.0) with sucrose (> 55%) [46], whereas LMP forms gels by the interaction of divalent cations, especially Ca^{2+}, between free carboxyl groups [47]. However, the DE represents only the ratio between methanol-esterified carboxyl groups and free carboxyl groups, whereas the MeO refers to the amount of methoxyl groups in a sample [48]. Therefore, the DE should not be assessed separately, as it does not represent the actual amount of methyl esterification, especially when the AUA content is low.

The DE of mango in this study ranged from 31.4 to 69.1% (table 3). Nahar et al. (2017) [34] found a value from 40.99 to 79.16% for Mango cultivars. Passion fruit in the present study had a DE ranging from 41.3 to 67.6%. Liew et al., 2014 [49] argued a value from 41.67 to 67.31% using citric acid at pH ranging from 2 to 4.5 and at LSR of 25ml/g. Pinheiro *et al*. (2008) [50] also reported the recovery of HMP from yellow passion fruit peels using citric acid. Kliemann et al. (2009) [53] have successfully recuperated LMP from yellow passion fruit wastes through acid extractions. DE of Bambara groundnut varied from 45.5 to 78.0%. These values were higher than of mango and passion fruit. These results demonstrated the possibility of obtaining of both HMP and LMP from the three individual sources.

Generally, the DEs of the individual sources increased when the pH and the LSR augmented except for Bambara groundnut where DE decreased with the LSR. According to Kliemann *et al*. (2009) [51], higher LSR of citric acid increased pectin DE. This finding is confirmed with mango and passion fruit pectins but is not in the case of Bambara groundnut pectin. Like the AUA content, the DEs of Bambara groundnut and its mixtures decreased when the LSR raised up.

The pectins obtained at low acidic condition (pH 1.5) can be considered as LMP because they had a DE inferior to 50% except for Bambara groundnut (<55%). At pH 2, all the pectins had a DE superior to 50% and can be classified as HMP. DE increased with increasing pH. The result was relevant to the previous study [32, 52]. The access of different values of DE at the two acidic condition (<55% at pH 1.5 and > 55% at pH 2) and the antagonism between mango/passion fruit and Bambara groundnut in the two condition of LSR offer the possibility of the production of interesting pectin in term of yield and in term of quality.

4.2. Optimization

4.2.1. Low Methoxyl pectin (LMP)

The results confirm the role of Bambara groundnut in the mixture as mentioned above. None of the plant sources match to the specifications because of the EW content (≥ 600). Bambara

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groundnut alone offers high level of EW at severe extraction conditions. Mixing leads to an improvement of the yield due to the antagonism between mango/passion fruit and Bambara groundnut during extraction. The optimum yield (40.3%) from mango-Bambara mix is higher than the published yields from conventional sources [10]. The co-existence of the two sources in the extractor permits the creation of a local condition that is favorable simultaneously in the improvement of the extraction yield [12] and in the preservation of the quality of the weak pectin against severe extraction conditions. Then, mixing is an appropriate method to the production of LMP in low acidic condition when the two plant sources present different comportment during extraction.

4.2.2. High Methoxyl pectins (HMP)

It was possible to obtain high yields and good qualities of different types of HMP thanks to the presence of Bambara groundnut in the mixes. Good qualities of slow set pectin (30.0%), medium set pectin (27.5%), and rapid set pectin (19.0%) were obtained from the MB mix. These results demonstrates again the advantage of mixing two plant sources when they have different comportment at the experimental conditions. MP mix was not excellent compared to the mango alone and the passion fruit alone because they had similar comportment during extraction. The yields above can compete those of the commercial pectins comprised between 15-30% (for citrus peel) and 10–15 % (for apple pomace) on a dry weight basis [10].

5. CONCLUSION

The EW, MeO, AUA contents and DE are important parameters to characterize pectins. The EW evaluates the weigth of pure galacturocic acid wich is correlated to the ability of pectin to form gel. The MeO and the DE are importants factors in controlliig setting time of pectins. They are utilised for the classification of pectin in two groups: LMP and HMP. The DE also allows to categorize the HMP in four types: slow set, medium set, rapid set and ultra-rapid set. The AUA content indicates the purity of pectins. It should not be less than 65%.

The extraction yield of pectins and the values of the previous experimental responses depended on the plant source, the mixing ratio, the pH and the LSR. Mango and passion fruit pectins had high extraction yields and high AUA contents. But they had feeble value of EW, MeO and DE especially in more acidic extraction. In the contrary, Bambara groundnut pectin showed moderate AUA content and showed appreciable values of EW, MeO and DE at low pH. As the MeO and the DE are influenced by the pH, the variation of pH offers the possibility of the production of both LMP and HMP. The antagonism comportment of mango/passion fruit and Bambara groundnut in different level of pH and LSR is profitable for the obtaining of high yielded-pectins of good quality. They can be achieved by varying the proportion of mixture, the pH and the LSR. This is possible due to utilization of MPV design which can be considered as an innovative and efficient method in the domain of pectin extraction.

Four classes of optimized pectins were found in this research: 40.26% of LMP derived from 31.31% of mango and 68.69% of Bambara groundnut, 32.00% of slow set pectin resulting from the mix of 56.6% of mango and 43.4% Bambara groundnut, 27.5% of medium set pectin obtained from 95.0% mango and 5.0% of Bambara groundnut, 19.0% of rapid set pectin available from 54.6% of mango and 45.4% of Bambara groundnut.

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