Influences of the pH, the liquid/solid ratio, and the mixing of the wastes of mango, passion fruit and Bambara groundnut on the extraction yield of pectin

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Abstract- *The aim of this study is to find alternative sources of pectin from three plant wastes mixtures, namely mango (Mangifera indica) fody variety, yellow variety of passion fruit (Passiflora edulis) and creamy brown-striped variety of Bambara groundnut (Vigna subterranea). A Mixture Experiments with Process Variables (MPV) design was applied. It is a combination of Scheffé's centroid mixture design and a full factorial design of the process variables (pH and Liquid/Solid ratio: LSR). The pH level was set at 1.5 and 2. The LSR level was fixed at 12.5ml/g and 15ml/g. Citric acid was used as acidifying agent. Pectin was extracted in a two-step sequential extraction at 80°C for 60 minutes. The plant sources, the mixing and the joint action of the pH and the LSR had significant effects on the production of high-yielded pectins. All the mixes showed more important yields than individual plant sources at the couple of pH 1.5/LSR 15ml/g. At this condition, an optimum yield of 47.7% can be achieved with the mix composed by 69.7% of mango peel and 30.3% of Bambara groundnut shell. The MPV design is an innovative method permitting the identification of the synergetic or antagonistic effect of mixing and the selection of the suitable extraction conditions favorable to high-yielded extraction. Farther characterization, optimization and classification of these pectins will be needed in order to assess their commercial aptitude.*

Keywords*- pectin, wastes mixtures, MPV design, extraction, yield*

1. Introduction

Population growth, industrialization, and urbanization have accelerated the generation of all kinds of wastes, causing serious environmental problems (soil, air and water pollution) [1]. The treatment and consumption of fruits and vegetables, for example, produces solid waste that is sometimes very high. Among many others, three types of waste are considered in this study. The Bambara groundnut (*Vigna subterranea)* produces 13 to 38% [2]. Mango (*Mangifera indica*) releases 30-50% of which 15-20% comes from its skin [3]. Passion fruit (*Passiflora edulis*) discharges 65 to 75% [1, 4].

Madagascar's mango production amounts to 150,000 - 200,000 tons per year [5]. Passion fruit production is around 500 tons [6, 7]. Bambara groundnut is a legume that can improve the bioavailability of phosphorus in ferritic soil. From a nutritional point of view, it is the richest in methionine, and lysine of all vegetables foods, although it has not generated much interest in research and development [8, 9]. National production is estimated at 2,600 tons [10].

Significant quantities of pectin and other high value-added bioproducts (polyphenols, essential oils, enzymes, etc.) can be extracted from the wastes of these plants before recycling them into other products: lignocellulosic materials, organic fertilizers, etc. [11].

Citrus peel and apple pomace are the two major sources of pectin most industrially exploited. But various fruits and vegetables by-products have been identified as potential sources of marketable pectin. Mango peel and passion fruit rinds' pectins were already studied by several authors. Bambara groundnut shell contains appreciable quantity of fiber (25.19%). Therefore, it may contain a good amount of pectin [12-14].

Pectin is a ubiquitous natural polymer found in the cell membranes of superior plants. Its wide and growing use as a hydrocolloid by the food industry is rapidly increasing [15]. Its traditional use as a gelling, thickening and stabilizing agent is complemented by its emerging use as a fat substitute and functional ingredient in the health sector [16]. It also has a place in beauty products, personal care (paints, toothpaste, and shampoos) and the pharmaceutical sector, including its new use as a nutraceutical ingredient [17]. In 2010, the European Food Safety Authority (EFSA) validated the use of pectin as a dietary supplement in reducing postprandial glucose responses. It is involved in regulating blood cholesterol levels and increasing satiety, leading to a reduction in energy intake [18]. Given its wide use and the continuous improvement of its extraction technique, pectin will soon emerge as the world's leading bio-economic product [19].

Previous studies showed that the extraction yields of pectin from mango peel reached from 13% to 37% [20-23]. Passion fruit rind yielded between 13% and 70% according to the authors [24-27]. Maphosa and Jideani (2016) found the presence of uronic acid in the insoluble dietary fiber of Bambara groundnut ranging from 6.7% to 10.6% [28]. The presence of uronic acid in the seed of this vegetable food and the presence of fiber in its shell [14] suppose the presence of pectin substance in the shell.

On another side, Christy et al. in 2014 published a best pectin yield (8.6%) by mixing three different types of waste (banana, citrus, and papaya) compared to the pectin yields extracted separately from the waste (6.4%, 6.8%, and 6.4% respectively) [1]. Was the highest yield from mixture published by these authors just a coincidence or is there a law or model that can explain this mixing effect? To answer this question, the three previous plant wastes: mango *fody* variety, passion fruit yellow variety, cream brown-striped Bambara groundnut were blended and were subject to pectin extraction using MPV method [29, 30].

2. Materials and Methods

2.1. Preparation of the plant sources

The mango peel from *fody* variety, the yellow variety of passion fruit rinds, the shells of creamy brown-striped Bambara groundnut were recovered from the markets of Fianarantsoa, a city situated at 400km from Madagascar's capital. Immediately afterward, these wastes were carried to the LPS laboratory in Antamponjina Fianarantsoa. They were first washed and then sorted before being weighed. Then, they were dried in an oven at 50°C until they reached 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 then pretreated twice with 85° ethanol at 70°C for 20 minutes under reflux to remove soluble ethanol impurities (sugars, pigments, etc.) [31]. 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 MPV design is a combination of a simplex-centroid mixture design [32] and a full factorial design [29-30]. The simplex-centroid mixture design was constructed with 2q+1 runs. Where q is the number of independent variables whose sum is 1 or 100%. It was provided by Minitab® 18.1 Software (Minitab Inc., State College, PA, USA). As there were three independent variables (mango, passion fruit, and Bambara groundnut), the design involved seven runs: the three individual variables with one component (1), the three binary mixes with two components in equal proportions (½, ½), and the central point with three components in equal proportions (⅓, ⅓, ⅓) (Figure 1).

The full factorial design of the process variables (pH and the Liquid/Solid Ratio: LSR) is illustrated in table 1. Each of the two variables had two levels (level - and level +). The pH values were set at 1.5 and 2. The LSR were fixed at 12.5ml/g (150ml/12g) and 15ml/g (150ml/10g). The resulting complete MPV design required 28 runs (Table 2). The experiment were repeated twice.

2.3. Pectin extraction

After pre-treatment, the alcohol-insoluble residues of each components were placed under same conditions (temperature, time, pH and LSR). Pectin substances were recovered using the technique of sequential two-step extraction published by Mohamed et al. (2016) [32].

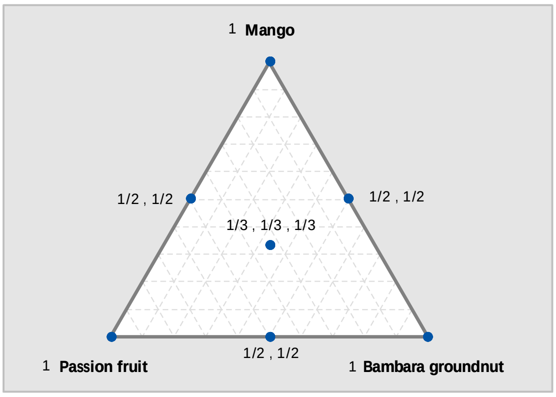


Figure 1: Simplex-centroid design of the three plants sources

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

The temperature and extraction time were set at 80°C and 60 minutes for each step [33]. Citric acid was used as acidifying agent. The pH of the extraction solution was previously adjusted at 1.5 or 2 using a Mettler Toledo pH tool. The previously treated mango, and/or passion fruit, and/or Bambara groundnut waste powders were weighed and mixed with 150ml of acidic solution. The LSR was set either at 12.5ml/g (150ml/12g) or 15ml/g (150ml/10g). The seven components were put into glass containers with the acidic solution. They were immersed in a thermostat bath set at 80°C and mixed from time to time with a spatula. After 60 minutes, they were filtered through a polyester cloth. The filtrates were cooled to room temperature. The residues were recovered and subjected to a second extraction under the same conditions. The achieved filtrates were mixed with the first ones and stored in a refrigerator at 4°C for one night. Double volumes of 96° ethanol were added to the refrigerated filtrates. Then, they were left for 1 hour to precipitate the pectins. The precipitates were washed twice with 70° ethanol and once with 96° ethanol. The purified pectins were stored in a refrigerator at 4°C overnight and then dried in a 50° oven until constant weight. Pectins before and after purification are illustrated in figure 2 and figure 3.

2.4. Pectin yields

The pectin yields were calculated using equation 1.

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

Where Y_{pec} (%) corresponds to 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 ethanol.

2.5. Regression model

The yields of pectins were subjected to multiple regression analysis using the MPV model (equation 6) resulting from the crossing of the Scheffé's quadratic model in equation 3 and the full factorial model in equation 4. The combined model is then found as the cross-product in equation 2.

$$Y_{x,z} = f(x) \times g(z) \quad (2)$$

The Scheffé's quadratic model for the 3 components is:

$$f(x) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (3)$$

The full factorial model is:

$$g(z) = \alpha_0 + \alpha_1 z_1 + \alpha_2 z_2 + \alpha_{12} z_1 z_2 \quad (4)$$

The combined model is:

$$Y_{x,z} = \beta_1(z) x_1 + \beta_2(z) x_2 + \beta_3(z) x_3 + \beta_{12}(z) x_1 x_2 \quad (5)$$

Where:

$$\beta_i(z) = \beta_i \times (\alpha_0 + \alpha_1 z_1 + \alpha_2 z_2 + \alpha_{12} z_1 z_2)$$

$$\beta_{ij}(z) = \beta_{ij} \times (\alpha_0 + \alpha_1 z_1 + \alpha_2 z_2 + \alpha_{12} z_1 z_2)$$

By multiplying the terms, as there are 3 terms in β_i and 3 other terms in β_{ij}, the full model contains 24 terms:

$$Y_{x,z} = \gamma_1^0 x_1 + \gamma_2^0 x_2 + \gamma_3^0 x_3 + \gamma_{12}^0 x_1 x_2 + \gamma_{13}^0 x_1 x_3 + \gamma_{23}^0 x_2 x_3 +$$
$$\gamma_1^1 x_1 z_1 + \gamma_2^1 x_2 z_1 + \gamma_3^1 x_3 z_1 + \gamma_1^2 x_1 z_2 + \gamma_2^2 x_2 z_2 + \gamma_3^2 x_3 z_2 +$$
$$\gamma_{12}^1 x_1 x_2 z_1 + \gamma_{13}^1 x_1 x_3 z_1 + \gamma_{23}^1 x_2 x_3 z_1 + \gamma_{12}^2 x_1 x_2 z_2 +$$
$$\gamma_{13}^2 x_1 x_3 z_2 + \gamma_{23}^2 x_2 x_3 z_2 + \gamma_1^{12} x_1 z_1 z_2 + \gamma_2^{12} x_2 z_1 z_2 +$$
$$\gamma_3^{12} x_3 z_1 z_2 + \gamma_{12}^{12} x_1 x_2 z_1 z_2 + \gamma_{13}^{12} x_1 x_3 z_1 z_2 + \gamma_{23}^{12} x_2 x_3 z_1 z_2 \quad (6)$$

Where: $\gamma_i^0 = \beta_i \times \alpha_0$, $\gamma_{ij}^0 = \beta_{ij} \times \alpha_0$, $\gamma_i^l = \beta_i \times \alpha_l$, $\gamma_{ij}^l = \beta_{ij} \times \alpha_l$, $\gamma_i^{lm} = \beta_i \times \alpha_{lm}$, $\gamma_{ij}^{lm} = \beta_{ij} \times \alpha_{lm}$.

$Y_{x,z}$ is the overall yield. In the first row of equation 6: x_i's are the linear terms of components, $x_i x_j$'s are the quadratic terms of components. In the second row: $x_i z_l$'s are the interaction terms of the individual sources with the pH or the LSR. In the third and last rows: $x_i x_j z_l$'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.

Figure 2: Pectins before purification at pH 1.5 and LSR 15ml/g

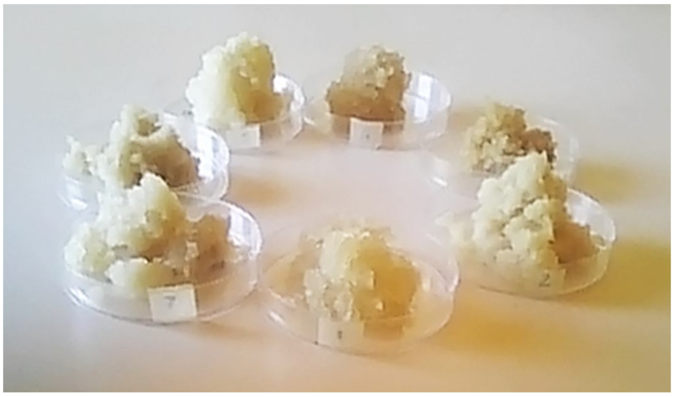


Figure 3: Pectins after purification at pH 1.5 and LSR 15ml/g

2.6. Results analysis

The model accuracy was checked by R^2, adjusted R^2, predicted R^2 and prediction error sum of squares (PRESS) [34]. The value of S or the "standard error of the model" was also estimated. A lower value of S indicates a better fitting model. The values of S, R^2 and adjusted R^2 indicate how well the model fits the observed data.

The values of PRESS and predicted R^2 are indicators of how well the regression model predicts new observations [35]. The regression model was validated by the ANOVA of the regression terms (p-value<0.05). The synergetic or antagonistic effect of mixing [36], the effects and the interaction effects of the process variables [34] were evaluated by the mean of the regression coefficients and visualized with the contour lines and the surface plots. The coefficients were analyzed with the Principal Components Analysis (PCA) and the Hierarchical Ascendant Clustering (HAC) methods provided by R 3.5.1 Software [37].

3. Results

3.1. Pectin yields

The results of the 28 runs are given in table 3. The yields ranged from 5.76±0.34% to 45.52±0.71%. They varied greatly with the pH and the LSR. They fluctuated from 5.76±0.34% to 12.79±0.02% at pH 2 and LSR 12.5 ml/g, from 13.77±0.33% to 29.16±0.21% at pH 2 and LSR 15ml/g, from 21.56±0.07% to 43.83±0.04% at pH 1.5 and LSR 12.5ml/g, and from 23.86±0.21% to 45.52±0.71% at pH 1.5 and LSR 15ml/g. They also diverse with regard to the component's type (individual or mixes). The effect of mixing and the effect of the pH and the LSR on the yields were important.

3.2. Validity of the model

The results in table 4 demonstrated the validity of the model which was very closely significant with low probability value ($p < 0.0001$) and the high F-value (3297.6). The high determination coefficient R-squared (0.9996), adjusted R-squared (0.9993) and predicted R-squared (0.9987) values, and the low value of S (0.32) indicate the accuracy of the model [35, 38]. An irrelevant p-value (0.737) of lack of fit performance and the low value of PRESS (9.94) signifying that the predicted models fitted well with the experimental data. These values suggested an excellent fit to the mathematic model in Equation 6, and the predicted values could reasonably represent the experimental values [29, 35].

Table 5 shows that the 23 terms of the model were significant (p-value < 0.0001). Only the term MB*LSR was not significant (p-value = 0.569). This term is not considered in the MPV model. The cubic term (MPB) was not significant (p-value = 0.731).

3.3. Validated model

The validated model is given in the regression below.

$$Y_{x,z} = 31.6x_1 + 23.1x_2 + 16.8x_3 + 5.1x_1x_2 + 18.0x_1x_3 + 3.4x_2x_3 - 11.8x_1z_1 - 5.8x_2z_1 - 7.1x_3z_1 + 3.4x_1x_2z_1 - 17.2x_1x_3z_1 + 3.9x_2x_3z_1 + 4.5x_1z_2 + 6.1x_2z_2 + 1.9x_3z_2 + 3.6x_1x_2z_2 + 5.9x_2x_3z_2 + 4.9x_1z_1z_2 + 1.7x_2z_1z_2 + 2.1x_3z_1z_2 - 6.7x_1x_2z_1z_2 - 13.9x_1x_3z_1z_2 - 4.2x_2x_3z_1z_2 + \varepsilon$$

Where x_1 = Mango, x_2 = Passion fruit, x_3 = Bambara groundnut,

z_1 = pH, z_2 = LSR

$Y_{x,z}$ = overall yield

3.4. Effects of the different terms of the model on yields

3.4.1. Individual sources and mixtures

The linear terms in table 5 are the mean of the individual yields from the whole experiments. The average yields of mango was the highest (31.6%), followed by passion fruit (23.1%) and Bambara groundnut (16.8%).

As for the mixtures, the MB mix had the highest coefficient (+18.0%), followed by MP and PB (+5.1% and +3.4% respectively). These coefficients determine the effects of the mixing on the yields. As they are positives, it proves the existence of synergetic effect of mixing on the extraction yield particularly with MB mix.

Table 3: Yields of Pectins at the experimental conditions

Run	Mix	pH	LSR	Average yield	Standard deviation
1	M	1.5	15	42.90	0.58
2	P	1.5	15	34.21	0.83
3	B	1.5	15	23.86	0.21
4	MP	1.5	15	40.84	0.06
5	MB	1.5	15	45.52	0.71
6	PB	1.5	15	31.39	0.01
7	MPB	1.5	15	41.10	0.26
8	M	2	15	29.16	0.21
9	P	2	15	25.06	0.06
10	B	2	15	13.77	0.33
11	MP	2	15	28.04	0.33
12	MB	2	15	18.37	0.21
13	PB	2	15	21.59	0.41
14	MPB	2	15	22.52	0.71
15	M	1.5	12.5	43.83	0.04
16	P	1.5	12.5	24.37	0.03
17	B	1.5	12.5	24.09	0.03
18	MP	1.5	12.5	31.26	0.35
29	MB	1.5	12.5	39.05	0.08
20	PB	1.5	12.5	21.56	0.07
21	MPB	1.5	12.5	30.92	0.01
22	M	2	12.5	10.41	0.02
23	P	2	12.5	9.63	0.04
24	B	2	12.5	5.76	0.34
25	MP	2	12.5	12.79	0.02
26	MB	2	12.5	11.51	0.02
27	PB	2	12.5	8.96	0.06
28	MPB	2	12.5	12.11	0.27

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

Table 4: Model accuracy

	F-value	p-value	R^2	R^2 adjusted	R^2 predicted	Lack of fit	S	PRESS
Value	3297.6	<0.0001	0.9996	0.9993	0.9987	0.74	0.32	9.94

3.4.2. Effect of the pH

The interaction terms with the pH were significant for all components. With individual sources, the decrease in pH from 2 to 1.5 provided a gain of 11.8% for mango, 7.1% for Bambara groundnut and 5.8% for passion fruit. With mixtures, the pH produced a gain or a loss of yield according to the plant types composing the mix. The lessening of pH contributed to a gain of 17.2% for MB mix. In contrary, passion fruit mixes did not support the decrease of pH. It caused a loss of 3.9% for PB mix and a loss of 3.4% for MP mix.

3.4.3. Effect of the LSR

The interaction terms with the LSR were significant except for MB mix. With individual sources, the coefficient reached up to 6.1%

for passion fruit, 4.5% for mango and 1.9% for Bambara groundnut. With the binary mixes, the effect of the LSR also differed according to the type of mixture. It increased the yield of passion fruit mixes (+5.9% for PB, and +3.6% for MP) but it was not significant for MB mixture.

Table 5: Model coefficients

Elements of the model		Terms	Coefficient	p-value
Component only	Linear terms	M	31,644	*
		P	23,083	*
		B	16,819	*
	Quadratic terms	MP	5,071	<0,0001
		MB	18,017	<0,0001
		PB	3,396	<0,0001
	Cubic term	MPB	-2,01	0.731
Interaction of the components and the process variables	Interaction terms with pH	M*pH	-11,824	<0,0001
		P*pH	-5,767	<0,0001
		B*pH	-7,115	<0,0001
		MP*pH	3,385	<0,0001
		MB*pH	-17,235	<0,0001
		PB*pH	3,91	<0,0001
	Interaction terms with LSR	M*LSR	4,526	<0,0001
		P*LSR	6,103	<0,0001
		B*LSR	1,901	<0,0001
		MP*LSR	3,567	<0,0001
		MB*LSR	0,621	0,425
		PB*LSR	5,875	<0,0001
	Interaction terms with pH and LSR	M*pH*LSR	4,936	<0,0001
		P*pH*LSR	1,663	<0,0001
		B*pH*LSR	2,111	<0,0001
		MP*pH*LSR	-6,74	<0,0001
		MB*pH*LSR	-13,92	<0,0001
		PB*pH*LSR	-4,232	<0,0001

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

3.4.4. Interaction effect of the pH and the LSR

The Interaction effects of the pH and the LSR (z_1z_2) on the yields were significant in all cases. The coefficients were positives with the individual terms (+4.9% for mango, +1.7% for passion fruit and +2.1% for Bambara groundnut). But, they were negatives with the mixes' terms (-13.9% for MB, -6.7% for MP, and -4.2% for PB). This sign inversion means that the interaction effect of the couple pH/LSR with the individual sources and with the mixes were contradictory. The positive interaction effect of this couple with individual sources was noticeable at the conditions where the sign of the couple was positive. These conditions are satisfied at pH 1.5 and LSR 12.5ml/g or at pH 2 and LSR 15ml/g. In the contrary, the positive interaction effect of the couple pH/LSR with the mixes was observable at the conditions where the sign of the couple were negative. These conditions were fulfilled at pH 1.5 and LSR 15ml/g or at pH 2 and LSR 12.5.

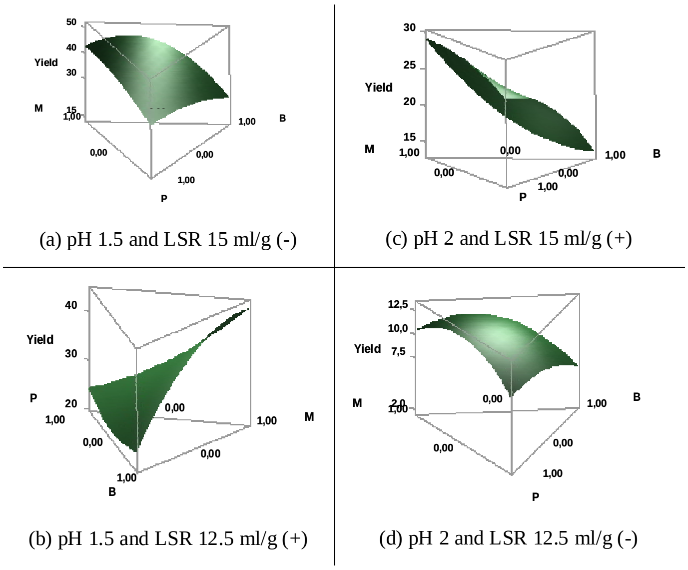
It means that the yields of the mixes were higher than the yields of the individual sources when the sign of the couple pH/LSR was negative, i. e. at pH 1.5 and LSR 15 ml/g (Figure 4a), or at pH 2 and LSR 12.5 ml/g (Figure 4d). The interaction effect of the couple pH/LSR with the mixes lessened their yields at pH/LSR (+), i.e. at pH 2 and LSR 15 ml/g (Figure 4c), or at pH 1.5 and LSR 12.5 ml/g (Figure 4b), but the individual effect of the pH and the LSR also contributed to the mixes' yields at these conditions.

At pH 1.5 and LSR 15 ml/g (Figure 5a), the extraction yields were maximum. They ranges between 20% and 48%. The maximum yield (>45%) belonged to the area of MB mix where the proportion of mango was superior to 50%. The optimum value reached up to 47.7%. It can be extracted from 69.7% of mango and 30.3% of Bambara groundnut.

At pH 1.5 and LSR 12.5 ml/g (figure 5b), the yield varied between 20% and 45%. Mango offered the maximum yield.

At pH 2 and LSR 12.5 ml/g (Figure 4c), the yields decreased between 12% and 30%. The yield of mango and passion fruit were superior to 25%. The maximum yield (29%) belonged to mango.

At pH 2 and LSR 15 ml/g (Figure 4d), the yields were minimum and lessened between 6 and 15%. The maximum yield (13%) was found in the area of MP mix. The mixes' yields were higher than the individual yields.



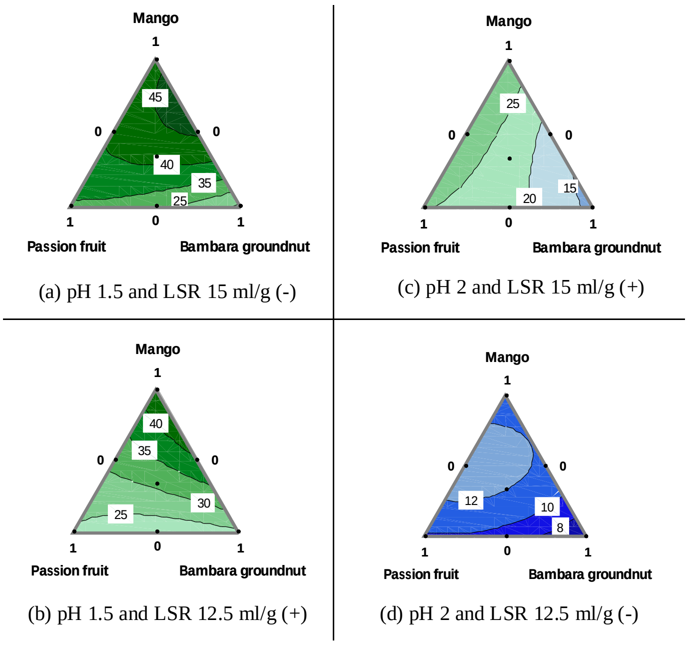
(a) pH 1.5 and LSR 15 ml/g (-)

(c) pH 2 and LSR 15 ml/g (+)

(b) pH 1.5 and LSR 12.5 ml/g (+)

(d) pH 2 and LSR 12.5 ml/g (-)

Figure 4: Surface plot of the yield according to the pH and the LSR



(a) pH 1.5 and LSR 15 ml/g (-)

(c) pH 2 and LSR 15 ml/g (+)

(b) pH 1.5 and LSR 12.5 ml/g (+)

(d) pH 2 and LSR 12.5 ml/g (-)

Figure 5: Contour plot of the yield according to the pH and the LSR

3.5. Interpretation of the coefficients

3.5.1. Principal Component Analysis (PCA)

The PCA of the coefficients in figure 6 illustrates that the gain or the loss of components' yields were associated to the pH, and the interaction of the couple pH/LSR. Axis 1 is correleted to the pH (p-value = 0.0076), and axis 2 is correleted to the couple pH/LSR (p-value = 0.0142). The improvement of components' yield was correlated negatively to the pH and positively to the interaction of the couple pH/LSR. Furthermore, the factor map shows that there was no correlation between the components and the LSR. However, the LSR is correlated to the pH and to the couple pH/LSR. It means that the amount of water during the extraction did not interfere directly to the gain of yields but indirectly from its combination with the pH.

3.5.2. Cluster map

The axes of the cluster map in figure 7 were built with the coefficients, i.e. the gain or the loss of yields. The position close to the center of the map means that the component did not profit any gain of yield. The more the components move away from the center, the more their yields get significant points. The right direction along to axis 1 means that the gain of yield was due mainly to the principal effect of the pH and indirectly to the LSR. So, the important yields of PB and MP mixes in figure 4c did not emanate from the interaction of the couple pH/LSR but from the main effects of the pH and the LSR separately.

As the couple pH/LSR is correlated to axis 2, its direction from the top indicates that increasing of components' yield was due to pH/LSR (+).The opposite direction means that the gain of yield derived from the pH/LSR (-). Moreover, the individual components are situated above axis 1, the mixes are positioned below. So, this factor map demonstrates that the very important yield's improvement of MB mix was due primarily to the mixing, secondly to the low pH (according to axis 1), and finally to the interaction of the couple pH/LSR (along with axis 2).

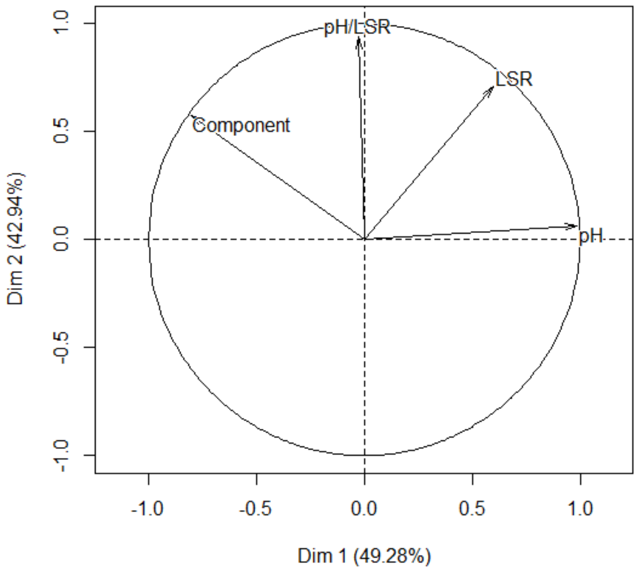


Figure 6: PCA of the coefficients

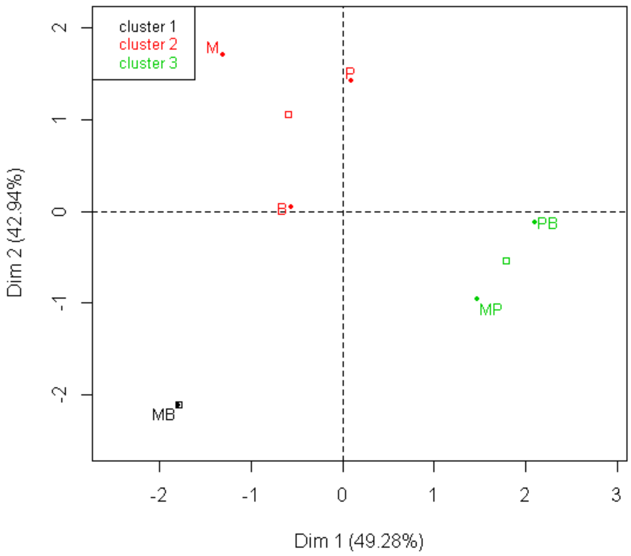


Figure 7: Cluster map

4. DISCUSSIONS

4.1. Regression model

The MPV model and the inside Scheffé's quadratic model was validated by the analysis of variance. The hypothesis of synergistic effect of mixing on pectin yields was proven with the three types of mixture.

All the scenarios (Mango-Bambara groundnut, Mango-Passion fruit, and Passion fruit-Bambara groundnut) showed positive regression coefficients. The result published by Christy *et al.* (2014) [1] was therefore not a coincidence, the mixing of the three plant sources (banana, citrus and papaya) would provoke the increasing of the mix's pectin yield.

The interaction terms of the pH and/or the LSR with the individual sources and the mixes in the present research were significant with high coefficient values. The importance of these interactions depended on the plant sources. The interaction effects of the couple pH/LSR with individual plant sources were frequent with the classical RSM method frequently used in pectin extraction. They varied greatly with the plant sources.

Except the results published by Maran et al. (2015) [38] presenting an appreciable value of pH/LSR interaction with mango peel with RSM method (coefficient = -1.21, p = 0.0248), most of the authors found little coefficient values.

Some of them are listed here: Amberalla peel (coefficient = 0.51 p= 0.008019) [40], *Helianthus annuus* head (coefficient = −0.20963, p<0.0001) [41], custard apple peel (coefficient = -0.082, p < 0.0001) [42], waste durian rind (coefficient = -0.057, p < 0.0001) [39], and Sweet potato residues (coefficient = 0.0005 p= 0.008019) [43]. These feeble coefficients suppose that this interaction effect was negligible. However, the results published here with MPV method highlighted the importance of these interaction effects particularly with the mixes. Thus, MPV is an innovative method which permit the detecting of new and interesting domain of researches for the improvement of pectin extraction.

4.2. Yields of the individual sources

4.2.1. Mango peel

As shown in the regression model, the average pectin yield of mango peel at the experimental domain reached to 31.6% (maximum 43.83%, minimum 10.41%). This percentage corroborates the previous results in the literature review varying from 13% from 37% [20-23]. Moreover, Nahar et al., (2017) [44] discovered different yields (17.31%, 20.62%, 25.53% and 30.43%) for four mango cultivars (*Kharsapat, Langra, Fazlee and Amrapali*) in a single extraction using H_2SO_4 with $(NH_4)_2SO_4$ buffer at pH 1.5. The average yield in the present research was higher than the *amrapali* cultivar due to the second extraction and the cultivar used (*fody*). The sequential extraction improves the extraction yield of pectin [32, 33]. Moreover, Yapo (2009) [33] confirmed that the use of citric acid gives less damaging effect on the pectin extraction.

4.2.2. Passion fruit rind

The average yield of passion fruit in this research attained 23.3% (maximum 34.21%, minimum 9.63%). This results were consistent with those published by the authors in the literature review oscillated between 13% and 70% [24-27]. With the same type of acidifying agent, Juan Carlos *et al.* (2010) [45] published a yield from 25 to 30%. Recently, Puspita *et al.* (2018) [46] found a maximum yield of 30.78% using hydrochloric acid.

4.2.3. Bambara groundnut shell

No result is yet published for the extraction of pectin from the shell of Bambara groundnut. In this research, the average yield of Bambara groundnut pectin reached 16.9% (maximum 25.9%, minimum 5.76%). It was very close to the percentage of pectin that can be extracted from apple pomace. Kumar and Chauhan (2010) [47] found that maximum extraction yields of pectin from pomace using diluted citric acid were 16.65% for Royal variety and 18.79% for Golden variety. This result was appreciable because the shell of Bambara groundnut can represent up to 38% of its total weight [2]. It confirms the otherness of Bambara groundnut over other leguminous with regard to dietary fiber content affirmed by Maphosa and Jideani (2016) [28]. Besides, Bambara groundnut begins to attract researchers, especially in the agri-food sector because of its high content of essential amino acids [48-50]. This is a good reason to exploit its by-product.

4.3. Yields of the mixes

In general, all the mixes offered yields higher than the mean of the pectin yields calculated from the individual yields. This confirms the hypothesis of the synergic effect of mixing on the yield of pectin. It was very significant with MB mixture (coefficient = 18.0%) but less significant with mixes involving passion fruit (coefficient = 5.1% for MP mix and 3.4% for PB mix). The significance of the mixing effect depends on the type of the plant sources composing the mix. It was due to the different comportment of the two plant sources at the experimental conditions. The presence of one plant source may create a favorable local condition which enhances or barricaded the extraction of the other. Synergism or antagonism between different types of wastes is frequent in the domain of the fermentation process for the production of biogas. Simultaneous presence of several types of residues in the co-digestion process may, due to significant synergistic interactions, improve the process [51-53], producing a higher methane yield than is obtained from individual fractions. Likely, as the recovery of pectin did not depend only on the heating step but also on the gel forming during alcohol precipitation, by the experimental condition (pH and LSR), native metallic ions in the plant sources may participate to the aggregation of pectin producing the augmentation of the yield.

At acidic condition, divalent cations as Ca^{2+}, Mg^{2+}, Zn^{2+}, Si^{2+}, Cu^{2+}, and Fe^{2+} can bind to pectin chains according to the "egg-box" model [12, 54-57]. Besides, Alabadan et al. (2005) [58] demonstrated that Bambara groundnut shell ash in concrete can partially replace ordinary portland cement because of the presence of high proportion of SiO_2 (33.36%), CaO (10.91%), MgO (4.72%), Fe_2O_3 (2.16%) in the ash. The abundance of the divalent cations (Ca^{2+}, Mg^{2+}, Si^{2+}, Fe^{2+}) in the Bambara groundnut shell may be the origin of the high synergetic effect of mixing mango and Bambara groundnut at low pH.

Moreover, MB mix gave its maximum yield at low pH contrarily to MP and PB mixes whose yields were maximum at high pH. This observation is similar to the results published by Chaliha et al. (2017) [59]. They explained that *Terminalia ferdinandiana* has a high level of calcium (273–287 mg/100 g DW). Presence of both calcium and pectin in the *T. ferdinandiana* tissue indicate that pectin from this source may exist in the 'eggbox' formation where the Ca^{2+} ions fit in the cavities formed by non-methoxylated galacturonic acid residues.

4.4. Effects of the process variables

4.4.1. Effect of the pH

In the case of individual sources, the decrease in pH from 2 to 1.5 enhanced the yield to 11.8% for mango, 7.1% for Bambara groundnut and 5.8% for passion fruit. Recently, with mango peel, Oliveira *et al*. (2018) [60] attained a yield ranging from 18.8 to 32.1% by varying the pH from 2.84 to 1.16. Dos Santos *et al.* (2017) [61], published a yield varying from 9.88 to 30.22% for the assisted ultrasound extraction of passion fruit with citric acid at pH ranging from 2.6 to 1.2. Hamidon and Zaidel (2017) [62] noted that a pH variation of 1.5 to 2 contributes to a significant yield decrease for sweet potato skin. While a pH change from 2 to 3 had little effect on this yield. This may be due to the breakdown of hydrogen bonds and ester bonds between pectin and plant cell caused by the action of temperature and low pH. The links between hemicelluloses and pectin are thus broken. It brings to the increase of the rate of pectin diffusion [63-66].

As for mixes, the effect of the pH differed according to the type of mixture. The decrease of the pH increased the yield of MB mix to 17.2% but produced a loss of 3.9% and 34% respectively for PB and MP. These results shows that the low pH is favorable for MB mix but unfavorable for passion fruit mixes particularly when the LSR was low.

At low pH, as the hydrogen ion concentration of the solution is increased, ionization of the carboxylate groups is repressed, i.e., the highly hydrated carboxylate group is converted into hydrated carboxylic acid groups. The loss of carboxylate groups is able to reduce the repulsion of the polysaccharide molecules which promotes the gelation properties of pectin giving more precipitated pectin at lower pH [67].

4.4.2. Effects of the LSR

Generally, the yields increased with the LSR. In the case of individual sources, the deviation rate amounted to 4.5% for mango, 6.1% for passion fruit and 1.9% for Bambara groundnut. These values indicates that the high LSR is more favorable for the pectin extraction of passion fruit. It does not support the reducing of LSR. Kulkarni and Vijayanand (2009) [68] arrived at the same conclusion. By varying the LSR from 10 to 40 ml/g, they were able to improve the extraction yield of passion fruit from 1.91 to 14.8%. The optimum was obtained at 30 ml/g. Maran *et al.* (2015) [38] stipulated the same observation with mango skin by varying the LSR from 5 to 20ml/g, the optimum yield attained 28.86%, the optimum LSR was 18ml/g. It was near the high level of LSR in the present research.

Guo *et al*., (2001) [69] affirmed that it was due to the fact that water absorbed the thermal energy of the heating and promotes the swelling of plant tissues. This swelling caused an increase in the contact area between the plant matrix and the solvent. This resulted a rupture of the cell membrane, releasing more pectin into the extraction medium. But the present finding stipulated that, the LSR did not contribute directly to the gain of yield. It acted indirectly with the pH. The ANOVA test proved that the LSR was not significant with MB mix (p-value = 0.569) which had the highest yield among the components during extractions (45.52±0.71%). Moreover, the highest yields for mango only and for Bambara groundnut only were obtained at low pH and low LSR (43.83±0.04% and 24.09±0.03 respectively). With regard to the yield, high LSR was only important for passion fruit and its mixtures at high pH. Thus, the plant sources, the mixing and the inseparable actions of the pH and the LSR were all important for the procurement of high yielded-pectin. This close interference is explained below.

4.4.3. Interactions between the pH and the LSR

There were two types of interaction of the pH and the LSR on yields: the positive correlation effect between pH and LSR (case of passion fruit and its mixtures), and the interaction effect of the couple pH/LSR (for all cases). Previously, Juan Carlos et al. (2010) published a high yielded passion fruit comprised between 25 and 30% using citric acid (pH = 2.3) at LSR 80ml/g. This result confirms that passion fruit needs high pH and high LSR to optimize its pectin yield.

About the interaction of the couple pH/LSR, Maran et al. (2015) [38] demonstrated the existence of significant interaction effect of pH/LSR (coefficient = -1.21, p = 0.0248) on the pectin yield of mango peel using microwave assisted extraction and RSM methodology. The highest pectin yield (28.86%) was achieved at pH 2.7 and LSR 18ml/g. The couple pH 2.7/LSR18ml/g was not too far from the couple pH 2/LSR15ml/g in the present research. The yield of pectin published by these authors increased with growing LSR up to 20 ml g at the same pH. However, the increasing of LSR was exceeded beyond 20 ml/g, the solution get saturated with the solute as well as higher solvent was negatively affected the mass transfer rate and barricaded the penetration of the pectin into the solution and decreases the extraction yield [70]. Besides, the present finding demonstrated that the LSR acted indirectly via the interaction of the couple pH/LSR. Interaction effect appears when the value of the couple pH/LSR is suitable. It seems that the favorable extraction condition needs a balance between the pH and the LSR. This balance is specific for each plant source. Thus, the significance of this interaction depends on the plant sources and may be boosted by the mixing. As example, Zaid et al. (2016) [71] achieved a highest pectin yield of 42.5% from dragon fruit (*Hylocereus polyrhizus*) peels at pH 1.5 and LSR 10ml/g with mild ultrasound, stirring and citric acid. This couple of pH1.5/LSR10ml/g using citric acid is close to the couple pH1.5/LSR12.5ml/g in which maximum yield of mango (43.83±0.04%) and maximum yield of Bambara groundnut (24.09±0.03%) were obtained in the present research using citric acid.

5. CONCLUSION

It is important to notice that MPV method is an innovative method applicable in the domain of pectin extraction. It allows at the same time the comparison of the extraction yield between different sources and between individual sources and the mixtures. This method presents two advantages: (*i*) the detecting of synergetic or antagonistic effect of mixing, (*ii*) the discovering of important correlation or interaction effects of process variables (pH and/or LSR) facilitating the choice of the suitable condition for the production of high yielded-pectins.

The yields were maximum at pH 1.5/LSR 15ml/g. The individual yields were lesser than the mixes' yields. There were significant synergetic effect of mixing and/or significant correlation or interaction effects of the pH and the LSR on the mixture's yields. Optimum yield (47.7%) can be extracted from the mix composed by 69.7% of mango and 30.6% of Bambara groundnut. The plant sources, the mixing and the joint action of the pH and the LSR were all important for the procurement of high yielded-pectin.

In order to produce marketable pectins fitting the standard specifications, the achieved pectins in this research will be characterized, optimized quantitatively and qualitatively, and classified.

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