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Pectin extraction from the *Moringa oleifera* pods and its application in a jam

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ABSTRACT: Pectin is an important polysaccharide in food because of its functional property. It has the ability to form gels in an acidic medium and in the presence of sugars. For this reason, it is used in the food industry as a thickening agent. In this research, pectin was extracted from both dry and green pods of *Moringa oleifera*. Firstly, the dry and wet moringa pods were characterized, obtaining humidity values 12.12 and 23.18%; ash 1.75 and 0.67%; proteins 20.62 and 5.91%; pH 6.15 and 6.86, density 0.91 and 0.18 g/mL respectively. In the extraction of pectin, the acid hydrolysis method was used, obtaining a 41% yield for the green pods at pH 2 and 30% for the dried ones at pH 3 at 90°C and 90 minutes in both cases. The pectin with higher yield was characterized physicochemically yielding 3.45% in content of methoxyl, 1.3% of ash, gelling time of 4 minutes, 15% in degree of esterification and 73.92% of galacturonic acid. A sensory analysis applied to the jam made with the pectin obtained rated as excellent. It is concluded that the pectin obtained is of low methoxy, slow gelation and high purity; it can be used to make jam, with good sensory acceptance.

Keywords: Acid hydrolysis, jam, *Moringa oleifera,* pectin.

INTRODUCTION

Moringa oleifera (moringa) is a tree of small size and accelerated growth, which generally reaches between 10 and 12 m height, with a scattered crown and pinnate leaves in three, currently valued for its leaves, roots, stems, flowers and seeds which has been studied and determined to be excellent for different uses. Many properties are attributed to all of the parts of this tree, beneficial not only to health but also to the environment (Gómez, 2013).

The main contributions made by moringa in terms of macro and micronutrients are found in the leaves, which, like fresh pods, show a considerable value of vitamin A in the form of β-carotenes, minerals (iron, potassium and calcium) and Vitamin C. In addition, dried and ground leaves have up to 30% protein on a dry basis, which is why the leaves are known to have higher sources of nutrients than pods. The seeds can contain up to 30 to 42% of oil, but also, the leftover cake contains 60% protein (Olson, 2011).

The pods are brown, three-sided, linear and sloping capsules with longitudinal grooves, usually 20 to 45 cm long, although sometimes up to 120 cm long, and 2 to 2.5 cm wide that give the appearance of a pod. A transversely cut shows a triangular section with several seeds arranged along (Garcia et al., 2013).

Pectins are polysaccharides of plant origin, heterogeneous, hygroscopic and soluble in acids and water, with gelling properties, stabilization of emulsions and nutritional fiber contribution (Aldana et al., 2011). It can be obtained from various plant resources and be of high or low degree of esterification or percentage of methoxyl and amidic pectin. Due to its degree of gelling capacity, it is one of the main products used for the production of of jams, compotes, sweets, jellies, bakery products, pastries, beverages and other foods, since it provides them with elasticity, structure and natural enhancement of the inherent flavor of the fruits desired by the manufacturer and consumer (Arellano and Hernández, 2013).

Pectins are recognized as natural additives that can be modified with the help of some chemical reactions. They can be intentionally added to some foods to improve their physical properties, taste, and preservation, among others; they help to restore certain foods that have a degraded texture because of conservation treatments, but they cannot increase their nutritional value (Araque and Moscoso, 2013). They have the property of forming gels in an acid medium, which together with polyhydrolyzed sugars are used in the food industry as a gelling and thickening agent (Abzueta, 2012).

Currently, Venezuela does not have companies that produce food additives, especially pectin (Arellano and Hernández, 2013), which contributes to the increase in costs due to imports. Because of this, the objective of this research is to extract pectin from the *Moringa oleifera* pod, so it can be used in the production of jam; this tree reproduces in great shape in the country, especially in the state of Falcón, Venezuela, and it is not given a significant use.

For the extraction of pectin, it is used acid hydrolysis, a method that is industrially applied. Thus, costs due to import of additives are minimized and is given a greater benefit to this easily accessible plant. It will also contribute to the construction of a productive independence and food sovereignty and security that guarantees the population's right to food based on social well-being and economic development based on laboratory-scale production models.

MATERIALS AND METHODS

Moringa oleifera pods, both green and in a ripe state, were obtained from trees located in the Carirubana municipality in the city of Punto Fijo in Falcón state, Venezuela. The selected samples were located in the North and Carirubana parishes of the city.

Physicochemical characterization of the *Moringa oleifera* pods

For the physicochemical characterization of the *Moringa oleifera* pod, they were subjected to a washing and disinfection process, and subsequently crushed. Next, the analyzes were carried out to determine the following parameters: Humidity (AOAC, 2019); Ashes (COVENIN, 1981); Proteins through the Kjeldahl method that is based on three process stages that are digestion, distillation and an evaluation; pH (COVENIN, 1979) and Density (COVENIN, 1998). The physicochemical analyzes of the pods were carried out in triplicate method. The reagents used were of analytical grade.

Pectin extraction through the acid hydrolysis method

The acid hydrolysis method was used, included manipulating variables such as pH, contact time and temperature. Where the raw material suspended in hot water with the necessary amount of a strong acid, in this case hydrochloric acid was used since Álvarez (2007) reports that it does not carbonize organic matter and is the least oxidizing compared to others available. Subsequently, the pectin precipitated in the presence of a primary alcohol; in this case, ethanol was used.

Eighteen hydrolysis was performed each with a sample of 10 g of crushed green pods and 200 mL of distilled water, adjusting the pH between 1 and 3 (Table 1). The temperature varied in two values: high (100°C) and low (90°C) and the contact time established in three times of 30, 60 and 90 minutes. At the end of the contact time, the aqueous solution was separated from the sample and allowed to cool for approximately two to three hours, followed by the addition of ethanol (ratio 1: 2), precipitating the pectin. Figure 1 shows the flow diagram of the acid hydrolysis process.

Finally, after 24 hours, the pectin was filtered from the aqueous solution using cloth filters. Pectin was placed in the oven for 24 hours at 70ºC in order to eliminate the humidity present and obtain a completely solid substance. The same procedure repeated for the dry pod samples (Table 2).

Physicochemical characterization of the pectin obtained

In this phase of the project, the pectin with the highest yield obtained through the acid hydrolysis method in the previous phase are physicochemically characterized. The most important characteristics that pectin must have are the indicative of its quality and for its use as a food gelling substance, their elements were analyzed.

For the characterization of the pectin obtained, the procedures carried out by Gamboa (2009) were used, which are: percentage of methoxyl was determined by the valuation method (Ferreira, 1976), the ash content by the general method of determining total ash (Hart and Fisher, 1984), for free acidity, the titration method used to reach the end (Lees, 1984). The degree of esterification and the galacturonic acid content present in the pectin established based on the Schultz titration method (Schultz, 1965). Finally, the time and gelling power (qualitative property) are properties of pectin that were estimated by measuring the time interval in which it took the gel to pectin and how consistently it could behave visually before gelling.

Table 1. Yields of acid hydrolysis with green pods.

pH	Contact time (min)	T (ºC)	Yields (%)
1	30	90	0.05
	60		0.7
	90		0.04
1	30	100	0.0
	60		0.0
	90		0.0
2	30	90	16.0
	60		37.0
	90		41.0
2	30	100	8.0
	60		10.0
	90		18.0
3	30	90	8.0
	60		23.0
	90		0.02
3	30	100	0.0
	60		0.0
	90		0.0

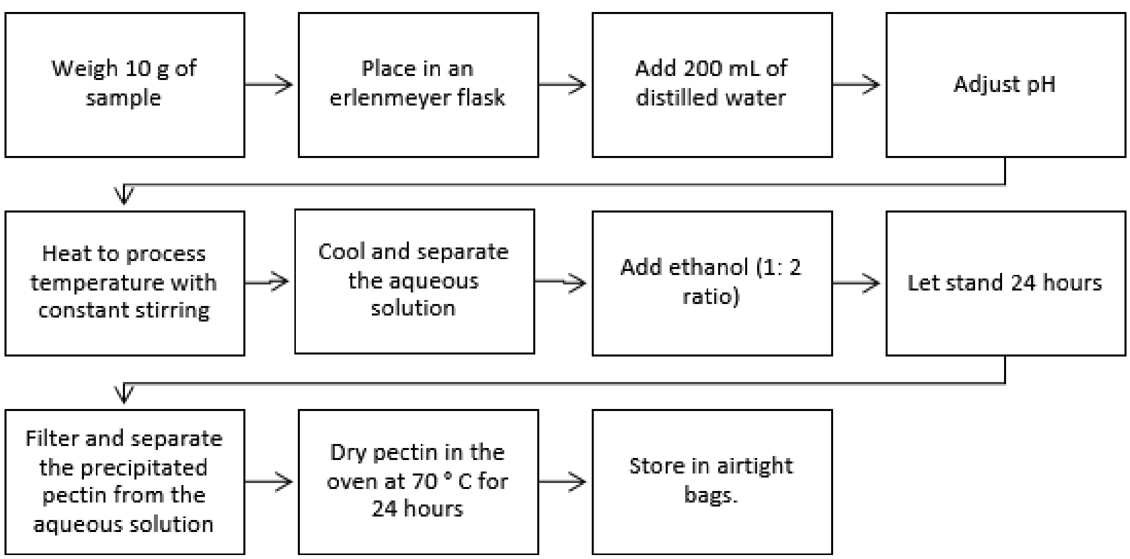

Figure 1. Flow diagram of the acid hydrolysis process.

Preparation of strawberry jam using the obtained pectin

At this stage, the jam made in order to evaluate the performance of the pectin obtained as a thickening additive. The preparation of the jam carried out through the procedure given by Coronado and Hilario (2001); finally, its organoleptic properties (odor, color, flavor, texture) evaluated through the execution of a sensory analysis.

The following ingredients used for the preparation: strawberry (300 g), sugar (300 g), pectin (0.49 g), water and citric acid. Initially, the strawberry was subjected to

Table 2. Acid hydrolysis yields with dry pods.

pH	Contact time (min)	T (ºC)	Yields (%)
1	30	90	0.0
	60		0.0
	90		0.0
1	30	100	0.0
	60		0.0
	90		0.0
2	30	90	0.0
	60		20.0
	90		25.0
2	30	100	0.0
	60		10.0
	90		15.0
3	30	90	0.0
	60		20.0
	90		30.0
3	30	100	0.0
	60		8.0
	90		10.0

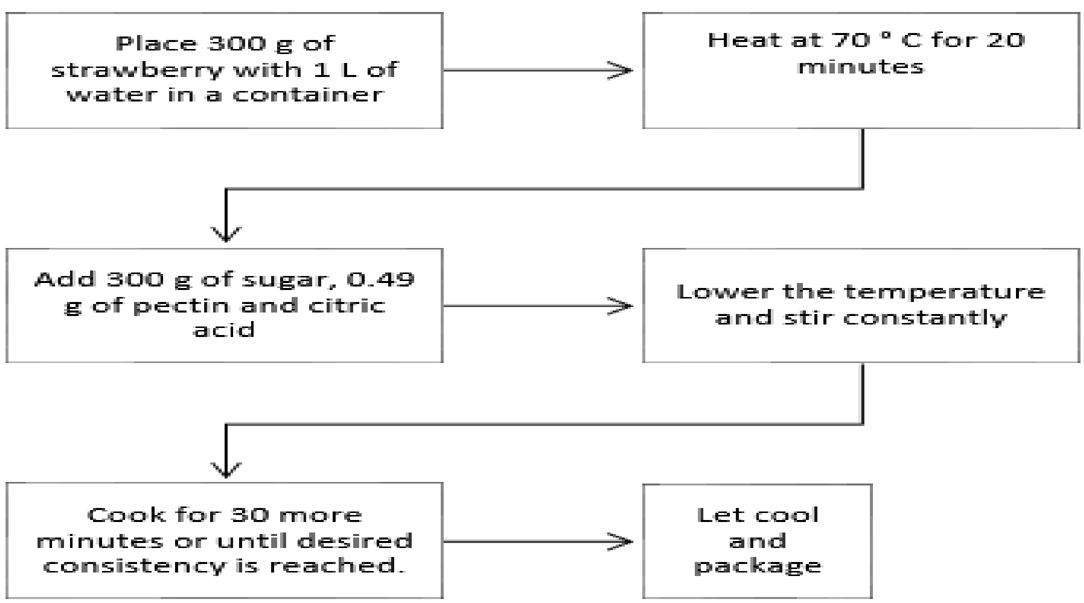

Figure 2. Flow diagram of the jam making process.

cooking process over medium heat (70ºC) with approximately 1 liter of water for 20 minutes, during which time its volume decreased, as time passed and the water evaporated. Gradually adding a prudent amount of water so that the pulp does not burn or stick to the walls of the container. After 8 to 10 minutes of the cooking time, the sugar, pectin and citric acid were added, maintaining constant agitation, without adding water and over low heat (65ºC), for approximately 30 minutes, reaching the desired consistency. Figure 2 shows the flow diagram of the jam making process.

Finally, its organoleptic properties (odor, color, flavor, texture) evaluated through a sensory analysis, where the preference, the degree of satisfaction and the acceptance of the jam made by the consumers were evaluated. For this, 50 samples of the processed jam taken and made available to consumers, the observations made were expressed using a checklist provided at the beginning of the evaluation.

Statistical analysis

All determinations were made in triplicate. Statistical analysis was carried out using Multifactor Analysis of Variance, with a significance value of $p<0.05$. Statgraphics Centurion XV software was used.

RESULTS AND DISCUSSION

In the physicochemical characterization, both the green and dry pods were evaluated. The percentage of humidity obtained was higher in the green pods, a value that almost doubled that of dry pods. For the ash content present, the values are considerably low and are close to those reported by Alfaro (2008). It should be noted that the amount of ash in food is inorganic residues remaining after the organic matter of the food has been destroyed in a muffle.

The protein content for dry pods was 21.8% and for green pods 5.9%, these results show similarity with those of Alfaro and Martinez (2008). Regarding the pH measurement, the obtained value is similar in both states (dry and green). This parameter (pH) is the factor that affect bacterial growth in food. On the other hand, it was possible to determine the density, which resulted in the green pods having a lower density than the dry ones; these results are summarized in Table 3.

The values of the proximal analysis varied according to what was found by other authors (Alfaro, 2008; Alfaro and Martinez, 2008), which possibly depends on genetic variability, degree of maturity, soil conditions, climate and the availability of light and water, among others (Alegria et al. 2005).

Pectin extraction by acid hydrolysis

By applying the acid hydrolysis method, it was possible to establish the conditions for the extraction of pectin from the green pods, the conditions at which the maximum yield was reached at pH 2 with a contact time of 90 minutes at 90ºC for a 41% yield.

The Pareto diagram (Figure 3) shows the significant influence of the temperature variable on the yield of pectin. This influence is negative, indicating that an increase in temperature will decrease the yield of pectin, the rest of the variables have no influence significant on performance. It also observed that the quadratic interaction of pH has a negative effect; this means that for higher pH values, lower pectin yield obtained.

The previous analysis verified in the ANOVA table (Table 4) where the p-value for each variable and its possible interactions reported in the last column and only those with this value less than 0.05 are significant on the performance of pectin for 95% confidence.

Figure 4 shows the main effects of the studied variables on the pectin yield. For temperature, the maximum yield observed at 90°C and this decreased as the temperature increased. For both time and pH, a minimum yield observed at 30 minutes and at pH equal to 1, while for a time of 90 minutes and pH equal to 2, maximum yield obtained.

Finally, from the results obtained, the following model can be used to predict the pectin yield of green pods through acid hydrolysis:

$$\text{Yield} = -105{,}018 + 0.243444 * \text{Temperature} + 1.07019 * \text{Time} + 128.439 * \text{pH} - 0.00389444 * \text{Temperature} * \text{Time} - 0.503833 * \text{Temperature} * \text{pH} - 0.00465648 * \text{Time}^2 - 0.0332083 * \text{Time} * \text{pH} - 19.0158 * \text{pH}^2$$

In the case of dry pods, the parameters under which the highest amount of pectin obtained were at pH 3 with 90 minutes of contact time at 90ºC, obtaining a 30% yield. This difference in pH to extract the pectin with respect to the green pods may be due to the ripeness of the fruits. In green pods, it is easy to break the fibers, while in mature pods it is more difficult to break the pectin-containing fibers, therefore a higher pH degree is required to achieve this. In addition, it is possible that they influence the content of minerals and other molecules, which benefit the formation of these compounds and the presence of structures that accompany pectin and that may affect the extraction method used (Alegría et al., 2005).

The Pareto diagram (Figure 5) shows the significant influence of time and pH variables on pectin yield, this influence is positive, indicating that an increase in them will increase pectin yield. The rest of variables have negative significant influence on performance. It was observed that the quadratic interaction of pH has a negative effect; this means that for higher pH values, lower pectin yield was obtained.

The analysis carried out previously verified in the ANOVA table (Table 5) where the p-value for each variable and its possible interactions reported in the last column and only those with the value less than 0.05 are significant on the performance of pectin for 95% confidence.

Figure 6 shows the main effects of the variables studied on the pectin yield. For temperature, the maximum yield was observed at 90°C and this decreased as the temperature increases. For both time and pH, a minimum yield was observed at 30 minutes and at pH equal to 1

Table 3. Determined physicochemical parameters.

Parameters	Dry pods	Green pods
Humidity (%)	12.12 ± 1.17	23.18 ± 3.26
Ash (%)	1.75 ± 0.39	0.67 ± 0.16
Proteins (%)	20.62 ± 1.18	5.91 ± 0.01
pH	6.15 ± 0.007	6.86 ± 0.007
Density (g/mL)	0.91 ± 0.00	0.18 ± 0.00

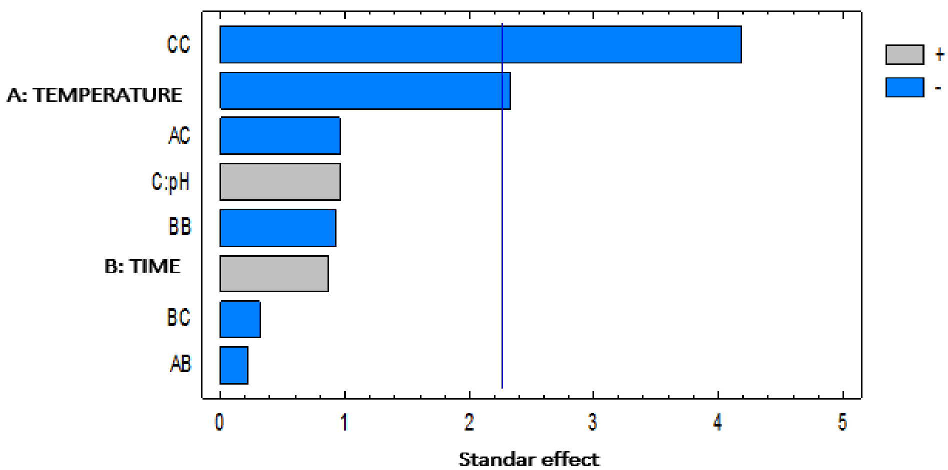

Figure 3. Pareto diagram for acid hydrolysis of green pods.

Table 4. ANOVA for green pod yield.

Source	Sum of Squares	Gl	Square Squared	F-Ratio	P-Value
A: Temperature	448.102	1	448.102	5.43	0.0448
B: Time	60.795	1	60.795	0.74	0.4131
C: pH	76.1544	1	76.1544	0.92	0.3619
AB	4.09501	1	4.09501	0.05	0.8287
AC	76.1544	1	76.1544	0.92	0.3619
BB	70.2523	1	70.2523	0.85	0.3803
BC	7.94011	1	7.94011	0.10	0.7635
CC	1446.41	1	1446.41	17.52	0.0024
Total error	743.012	9	82.5568		
Total (corr.)	2932.91	17			

while for a time of 90 minutes and pH equal to 3, the maximum yield was obtained.

Finally, from the results obtained, the following model can be used to predict the pectin yield of dry pods by acid hydrolysis:

Yield = -168,444 + 1.4889 * Temperature + 1.87222 * Time + 70.3333 * pH - 0.0166667 * Temperature * Time - 0.533333 * Temperature * pH - 0.00333333 * Time ^ 2 + 0.166667 * Time * pH - 6.0 * pH ^ 2

Physicochemical characterization of pectin obtained with higher yield through acid hydrolysis method

Regarding the physicochemical characterization of the pectin obtained with the highest yield in the previous stage (Table 6). The content of methoxyl is low, so they have the ability to form gels in the presence of divalent cations, commonly calcium (Gamboa, 2009).

In the case of total ash, it must be less than or equal to 10% according to the Food Chemicals Codex specifications

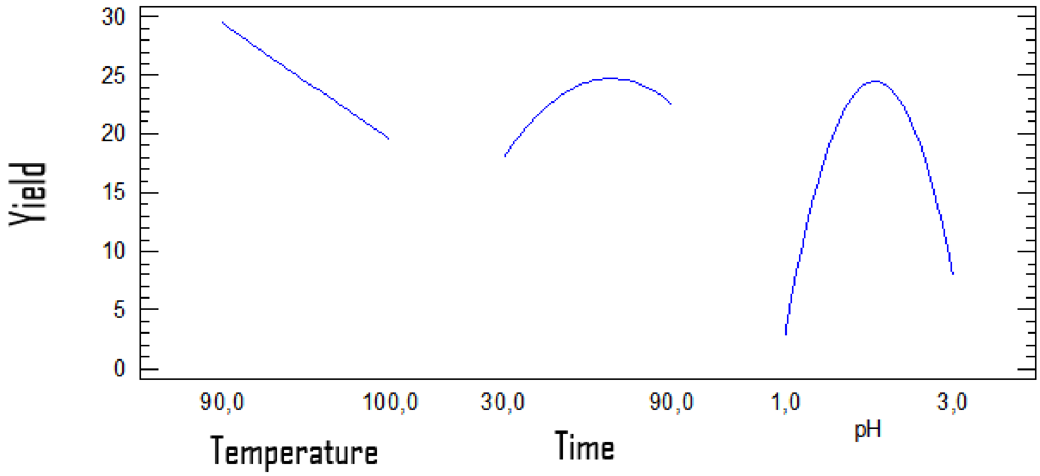

Figure 4. Pectin yield of green pods.

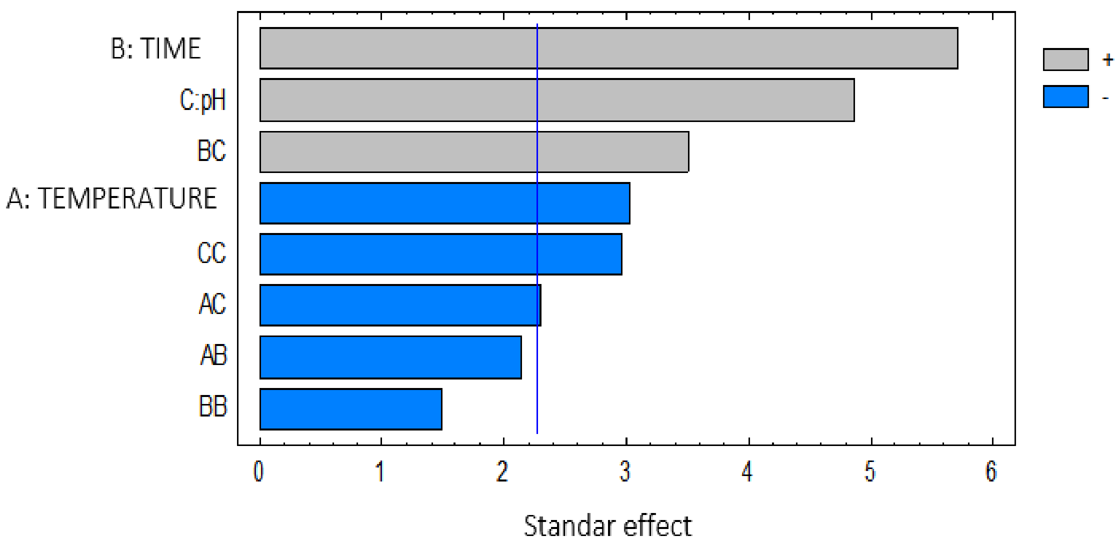

Figure 5. Pareto diagram for acid hydrolysis of dry pods.

Table 5. ANOVA for dry pod yield.

Source	Sum of Squares	Gl	Square Squared	F-Ratio	P-Value
A: Temperature	150.222	1	150.222	9.21	0.0141
B: Time	533.333	1	533.333	32.70	0.0003
C: pH	385.333	1	385.333	23.63	0.0009
AB	75.0	1	75.0	4.60	0.0606
AC	85.3333	1	85.3333	5.23	0.0480
BB	36.0	1	36.0	2.21	0.1715
BC	200.0	1	200.0	12.26	0.0067
CC	144.0	1	144.0	8.83	0.0157
Total error	146.778	9	16.3086		
Total (corr)	1756.0	17			

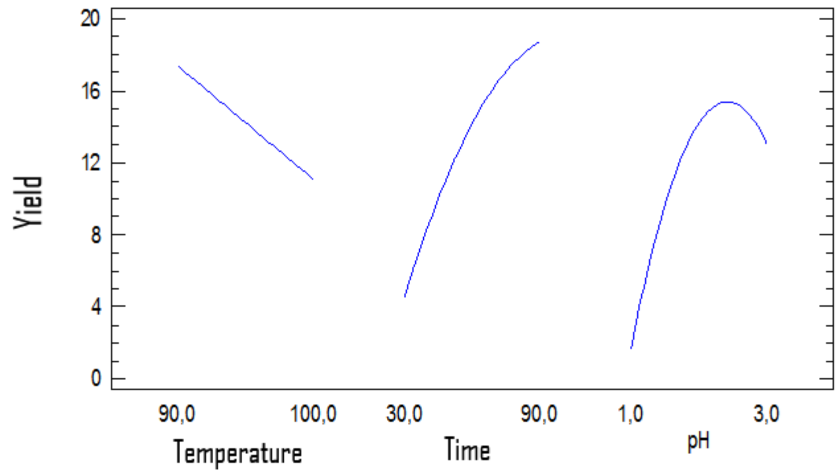

Figure 6. Pectin yield of dry pods.

Table 6. Characterization of pectin with higher yield.

Property	Property value
Methoxyl content (%)	3.45 ± 0.009
Ash (%)	1.3 ± 0.2
Gel time (min)	4.00
Degree of esterification (%)	15
Galacturonic acid content (%)	73.92 ± 3.05

for commercial pectins. However, Gamboa (2009) mentions total ash values ranging from 2.0 to 4.25% at a precipitation pH of 3.2 and 3.6. In the case of pectin obtained, this value was below that established by the Food Chemicals Codex.

Moringa pectin has a degree of esterification of 15% compared to that of commercial pectins, which is 81.5% (Moreno Quintero, 2014), making it slow gelling; this is also demonstrated with the time it took to reach the gel formation, which was equal to 4 minutes since the gelling speed decreases a lot with the degree of esterification (Vian, 2006).

It should be noted that the degree of esterification is the property that allows predicting the strength and type of gel, and the higher the degree of esterification, the greater the hydrophobic interactions. The rate of gelation influences the texture of the product and this tends to decrease when the degree of esterification decreases. In addition, slow gelling pectins are those that commonly used for the commercial production of jellies and reach maximum firmness at a pH of 3.0 to 3.15 (Gamboa, 2009).

The result obtained for the percentage of galacturonic acid was 73.92%. According to Pagán (1995), the richness of pectin in galacturonic acid (AGA) is related to the purity of the pectin obtained, hence the importance of quantifying it. However, galacturonic acid, being a sugar, an oxidized form of D-galactose, accompanied by neutral sugars such as, L-arabinose, L-ramosa, D-galactose and some impurities entrained in the extractions.

Preparation and organoleptic analysis of strawberry jam

The jam was made with the ingredients according to the Coronado and Hilario (2001) procedure. Because of the organoleptic analysis carried out, it was obtained for the odor that 43% rated it as like it a lot, while for color 68% of consumers evaluated it as like it a lot. For the flavor, 53% rated it as like it a lot, while the texture evaluated as like it moderately by 43% of consumers. Figure 7 graphically shows the results of the organoleptic analysis.

Conclusions

The pectin obtained is low methoxyl; it also has low ash content. It is slow gelling and is of high purity.

The gelling power and the degree of esterification confirm that the pectin obtained can be used in the manufacture of jams, dietary products, compotes, jellies, among others, since they have a positive behavior against the formation of gels. The jam made with the pectin obtained can be included in the food market since it has the main

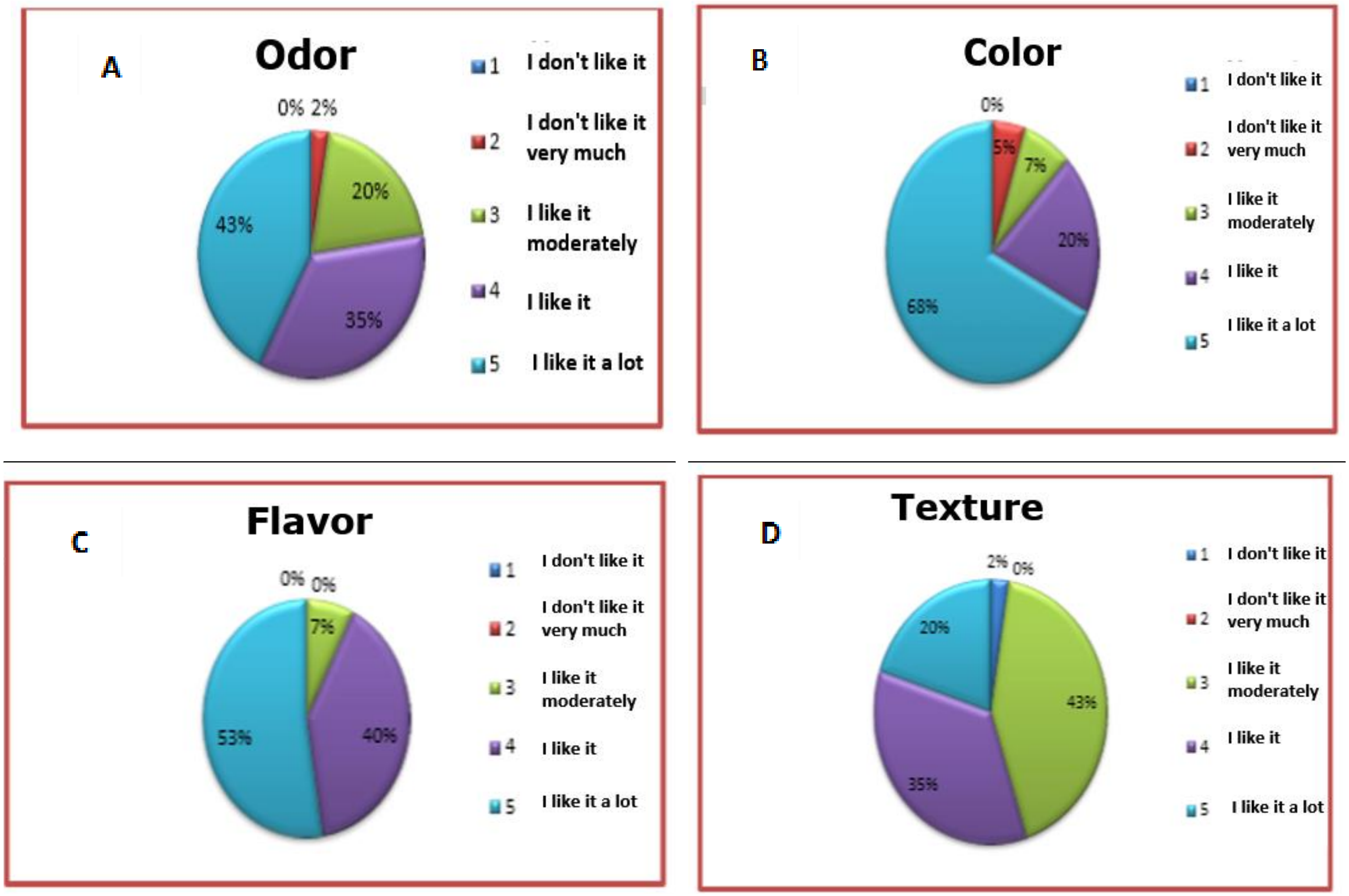

Figure 7. Acceptability results of the strawberry jam made with pectin from the *Moringa oleifera* pod: Odor (A), Color (B); Flavor (C) and Texture (D). Rating scale: 1 = I don't like it, 2 = I don't like it very much, 3 = I like it moderately, 4 = I like it, 5 = I like it a lot.

characteristics for consumer acceptance.

This research contributes to provide an alternative for obtaining this substance so important for the food industry from *Moringa oleifera* pods, and it is recommended to expand the studies on the subject for the production at an industrial level of pectin from the fruits of this tree.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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