Introduction

Pectin is a natural component of plants that is present in their cell walls and acts as a hydration agent and a binding material for cellulose. Although fruit pectin is used for jelly and jam gelification, it has become a valuable sub product in the processing of fruit juice, mainly obtained from the liquid and solid industrial waste of apples and citrus and from the juice of these fruits. Apples and citrus pulp contains approximately 20 to 30% dry extract, 1.5 to 2.5% pectin and 10 to 20% carbohydrates and the waste material represents approximately 4% of the fresh weight of the citrus (Arthey and Ashurst, 1997). Thus, apple juice-processing companies reuse both the liquid and solid wastes; the waste is dried and then used to produce pectin for canned vegetable products, jams, jellies and for the pharmaceutical industry (Linden and Lorient, 1996). Due to the increasing emphasis of pectin’s beneficial effects on human metabolism,
The importance of pectin has increased, creating a need to develop an appropriate technology to extract it from Pink Lady apples (*Malus pumila*), a cultivar known for its quality. Pink Lady apples are a rich source of pectin, presenting a content of nearly 30% in the primary cell walls, for use in food and pharmaceutical products (Arthey and Ashurst, 1997).

The aims of this study were to evaluate the characteristics of the pectin extracted from Pink Lady apples using standard chemical techniques, to classify the pectin (which was extracted from apples with a physiological maturity averaging 50% red-color coverage) based on the degree of esterification, to develop a standardized technique of pectin extraction and to evaluate the intensity of the sensory attributes and acceptability of the pectin obtained.

### Materials and methods

The raw material used was Pink Lady apples (*M. pumila*), planted 4.5 m x 3 m in a Romeral series of soils (very fine, sandy loam type), between 30-cm and 80-cm deep and under a system of micro-sprinkler irrigation, located in Fundo Marengo, km 5, Curicó province, Commune Los Niches (35 ° 01 ‘lat. S 71 ° 15’ long.), Maule Region, Chile (CIREN, 1997).

The temperatures averaged between a maximum of 27.5 ºC in January and a minimum of 4.1 ºC in July, with a frost-free period of 219 days and an average of 12 frosts per year and an average annual rainfall of 859 mm. The water deficit was 883 mm with a dry period of 7 months (Santibañez and Uribe, 1993).

The harvest of the apples occurred on April 9, 2007, using the indices of physiological maturity averages in the range of 45-55% red coverage, soluble solids 14.5 to 15 °Brix and a pressure of 18-19 lbs. They were then transferred to the Del Monte facility in Curicó for storage at a temperature of 1 ºC. Our study of the extraction of the pectin started in April 2007 and ended in July 2007.

The extraction of the pectin was performed in the laboratories of Agricultural Sciences and Basic Science, Universidad Católica del Maule, in both Curicó and Talca.

**Determining the degree of esterification (DE)**

The degree of esterification is an important chemical characteristic of pectin (Vorago *et al.*, 2009), which determines its behavior in the gelling process and its use in the food industry. The degree of esterification depends on having high or low methoxyl rates.

The degree of esterification was determined using the Schultz and Schweiger (1965) valuation method (compiled by Pagani, 1990), which consists of evaluating 10 mL of 1% pectin with 0.1 N NaOH, using 1% phenolphthalein as an indicator (valuation A). Then, 20 mL of 0.5 N NaOH were added and after 30 min, 20 mL of 0.5 N HCl were added to neutralize the excess NaOH to produce the valuation with 0.1 N NaOH (valuation B). The degree of esterification percentage was calculated using the following formula (Pagani, 1990):

\[
\text{DE} = \frac{B}{A+B} \times 100
\]

To determine the degree of esterification, three replicates per combination of treatment were created according to a 3 x 2 factorial model for the factors of the pH and the two heating times (Table 1).

| Table 1. pH values and heating times related to the evaluated treatments. |
|-----------------|-----------------|-----------------|
| Time (t min)    | Values of pH (P)|                  |
|                 | 0 (3.5)         | 1 (3.0)         | 2 (2.5)         |
| t₀ (60)         | T₀              | T₂              | T₀              |
| t₁ (90)         | T₁              | T₃              | T₁              |
| t₂ (180)        |                 |                 |                 |
**Pectin extraction process**

Simulated tests were performed for the extraction process; the Camejo *et al.* (1996a), Camejo *et al.* (1996b), Moya (2003), Devia (2003) and Ihl *et al.* (1992) methodologies were then adapted to adjust the analysis techniques based on the laboratory conditions.

To extract the pectin from the juice, samples of apples from the Del Monte Company were processed using a methodology similar to the one employed by Pagani (1990). The fruit was randomly obtained from the right, left, surface and center of the container and the selected Pink Lady samples were weighed and washed with water at room temperature using utensils previously sterilized with a chlorine solution. The apples were washed again with water, but at a temperature of 60 °C for 10 min to remove the hot water-soluble substances that could harm the pectin. Subsequently, the apples were subjected to an enzyme inactivation at 100 °C for 3 min to prevent the growth of microorganisms that could degrade the raw material.

In the grinding step, apples were ground using a blender and then subjected to filtration through a cloth or canvas. Once the juice was obtained, the °Brix, pH and acidity were measured using a 300 mL sample, according to the AOAC official methods of analysis (1990) and the juice was acidified with 99.8% citric acid solution to pH 3.0 and 2.5; the pH was 3.5 with no acidification. The juice was heated at a constant temperature of 90 °C for 60 or 90 min. The juice from each treatment was then filtered (at a temperature of 90 °C) using a cloth or canvas and allowed to cool to 20 °C.

The pectin in 250 mL of filtered juice was then precipitated with 250 mL of 96% ethyl alcohol for 30 min, when the separation of the pectin gelatinous clot was visible. The precipitated pectin was then separated under vacuum using Whatman 1 filter paper and a filter fabric or cloth placed on a funnel inserted into a Kitazato flask.

The extracted pectin was weighed and placed in a watch glass (5 g was retained for the esterification analysis) and transferred to a dessicator, which contained H$_2$SO$_4$, to act as a desiccant to remove the water from the pectin; the dessicator was connected to a vacuum pump for 7 min to remove the oxygen. The desiccator with the obtained sample was then placed in a dehydrator oven at a constant temperature of 45 °C for 20 h to obtain a sufficient drying and dehydration of the samples (Figure 1).

Once dry, the amount of pectin from the original sample was determined.

The experimental unit was a pectin solution diluted to 1%, corresponding to 5 g of pectin per treatment and a total of 90 g of pectin. The results were expressed in grams of dried pectin and degrees of esterification and were subjected to an analysis of variance (ANOVA) and multiple comparisons using the Tukey test (P≤0.05) when significant differences existed.

**Dehydrated pectin**

Several drying tests were carried out using temperatures between 40 and 50 °C and times between 5 to 20 h in a conventional oven. To improve the drying process, sulfuric acid (H$_2$SO$_4$) was used as desiccant. In the tests carried out without sulfuric acid, the pectin samples did not show dehydration, but the tests that used it showed an evident dehydration of the samples.

**Dehydrated pectin sensory analysis**

This evaluation was conducted in the laboratory of Agricultural Sciences at the Universidad Católica of Maule, Campus Nuestra Señora del Carmen, Curicó. The attributes of the extracted pectin were measured using an organoleptic analysis and a product acceptability evaluation by the 13 panelists or judges who were randomly selected and trained for the procedure.
The dried pectin samples of each treatment were given to each of the 13 panelists who evaluated approximately 0.5 g of each sample, which were placed in a plastic bag and identified with a letter from A through F, then placed on table in a white porcelain plate along with a glass of water, the sensory evaluation and acceptability (Stone and Sidel, 1993).
product, using another chart called structured verbal-numerical scale with scores, varying from 1 to 7 points (Meilgaard, 1999).

The results were analyzed using a 3 x 2 factorial model as an experimental design for the three pH factors and the two heating times, with three replicates per treatment combination.

An experimental block model design was applied to the sensory analysis and acceptability results. An analysis of variance (ANOVA) with multiple comparisons was performed; when significant differences were found, the Tukey test (P≤0.05) was used.

Results and discussion

Physical and chemical analysis

The apple juice analyses of quality, before the pectin extraction, are presented in Table 2.

Extracted pectin gel

The method used for the pectin extraction of Pink Lady apples was adjusted and standardized according to the study site.

A total of 526.06 g of pectin gel was obtained after the treatments, which showed a slight difference in the color and extracted quantities. The extraction at pH 2.5 (the T₄ and T₅ treatments) resulted in a light apricot gel color, whereas the pH 3.0 (the T₂ and T₃ treatments) and pH 3.5 (the T₀ and T₁ treatments) produced gels with a dark apricot color. Because 90 g of pectin was removed from the total for the degree of esterification analysis, 436.06 g of pectin was available then for the dehydration and 61.72 g of dried pectin was obtained after 20 h at 45 °C in a dehydrator. The T₁ treatment (pH 3.5 for 90 min) resulted in the highest amount of dried pectin (4.47 g, equivalent to 7.25%); therefore, this pH and heating time were the best for the pectin extraction from Pink Lady apples. The opposite occurred with the T₀, T₂ and T₃ treatments, with 60 min of heating, in which smaller amounts of dehydrated pectin were produced: 3.05 g (4.24%), 2.39 g (3.88 %) and 3.34 g (5.41%), respectively.

Degree of esterification (DE)

According to Figure 2, the treatments that had high DE (higher than 50%) were T₀ (pH 3.5 for 60 min) and T₁ (pH 3.5 for 90 min) at 68.27 and 65.92%, respectively; no significant differences were found between them. This is consistent with the work described by Wehr et al. (2004). These results are also consistent with the tests conducted by Adossio et al. (2005), Camejo et al. (1996a), Camejo et al. (1996b) and Pagani (1990), who obtained the highest degree of esterification (70.3%) at pH 3.0 for the fruit pectin extracted from passion fruit and also lemons. In addition, the percentages of pectin extracted from T₀ (4.24%, 3.05 g) and T₁ (7.25%, 4.47 g) treatments were higher than those obtained by Vasquez et al.

Table 2. Average values of the analytical determination made from the mashed apples.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble solids (°Brix)</td>
<td>15.2</td>
</tr>
<tr>
<td>pH</td>
<td>3.5</td>
</tr>
<tr>
<td>Acidity (g of citric acid anhidre/100 mL juice)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Average values of 10 determinations.

Figure 2. Degree of esterification obtained under different treatments. Means without a common letter are significantly different according to Tukey’s test at a significance level of 0.05. T₀: pH 3.5 for 60 min; T₁: pH 3.5 for 90 min; T₂: pH 3.0 for 60 min; T₃: pH 3.0 for 90 min; T₄: pH 2.5 for 60 min; T₅: pH 2.5 for 90 min.
(2008), who reported a degree of esterification of 12.72% from banana peel.

In all of the other treatments, the degree of esterification rates decreased as the pH was lowered and the heating time increased. Therefore, the T₄ (pH 2.5 for 60 min) and T₅ (pH 2.5 for 90 min) treatments presented significantly lower degree of esterification values, at 26.37 and 28.77%, respectively. However, the results were higher than those obtained by Vasquez et al. (2008). Regarding the pH, Pagani (1990) showed that increasing the acidity leads to changes in the degree of esterification, having no effect on the heating time (Wehr et al., 2004).

However, other authors, including Arthey and Ashurt (1997), have indicated that the degree of esterification decreases by lowering the pH and increasing the heating time, causing a deesterification of HM pectins and transforming them into weakly methylated pectins (LM); this coincides with the findings in the present study because the degree of esterification obtained at 60 and 90 min of heating was similar for the T₂, T₃, T₄ and T₅ treatments.

According to the analyses conducted in this study, the pectin extracted from apples would be within the range of HM pectins because, in most of the treatments, a degree of esterification higher than 50% was obtained, indicating that the pectin is suitable for use in the manufacture of jams, jellies and preserves due to its gelling property (Kim et al., 2008). A greater amount of pectin was obtained at a lower pH, but this treatment also produced a lower quality pectin due to its low degree of esterification. However, the T₁ treatment (pH 3.5 for 90 min) differed with respect to the T₂ and T₃ treatments, as it resulted in a higher amount of pectin (4.47 g).

The T₀, T₁, T₂ and T₃ treatments produced samples with degrees of esterification higher than 50%, which are classified as highly methylated pectins.

**Dried pectin**

The preliminary results were not satisfactory, as the samples had inadequate drying and a dark-brown caramelization.

A total of 61.72 g of dried pectin was obtained. The T₁ treatment (pH 3.5 for 90 min) presented a significantly higher quantity of dried pectin (4.47 g) when compared to the T₂ treatment (Figure 3).

![Figure 3. Dehydrated pectins in (g) and the percentage obtained under different treatments. Means without a common letter are significantly different according to Tukey’s test at a significance level of 0.05. T₀: pH 3.5 for 60 min; T₁: pH 3.5 for 90 min; T₂: pH 3.0 for 60 min; T₃: pH 3.0 for 90 min; T₄: pH 2.5 for 60 min; T₅: pH 2.5 for 90 min.](image)

The results obtained under the conditions in T₁ coincided with Camejo et al. (1996a), Camejo et al. (1996b), Pagani (1990) and Muhidinov et al. (2010), in which only 4.47 g (7.25%) of pectin was obtained in 90 min of heating time. However, this treatment resulted in a pectin extraction percentage that was lower than that reported by Vasquez et al. (2008), who obtained a pectin extraction of 15.7% at a pH of 2.5 and 2.0.

According to Pagani (1990), by increasing the extraction time from 60 to 120 min, under conditions of pH 1.0 to 2.5, the percentage of pectin obtained increased from 2.95 to 17.63%, reaching a maximum performance value of 8%, which began to decline up to 6%, most likely due to the pectin degradation when the extraction time was increased.
This could be due to two processes that are generated: the solubilization of pectin, which changes from a solid phase to an acidic solution until the substrate pectin is exhausted and the hydrolysis of soluble pectin during degradation, resulting in a decrease of extracted pectin yields at longer incubation times.

The $T_0$, $T_2$ and $T_4$ treatments, which used 60 min of heating, produced the lowest amounts of dried pectins, 3.05 g (4.24%), 2.39 g (3.88%) and 3.34 g (5.41%), respectively. These smaller amounts of pectins extracted would be caused by the lower extraction time that likely failed to dissolve the pectin in tissue completely.

The amounts of pectin extracted in $T_0$, $T_2$ and $T_4$ were theoretically superior to the tests by Devia (2003), who obtained 3.10% pectin from orange fruit, which is a lower percentage than the values obtained by Adossio et al. (2005), Camejo et al. (1996a), Camejo et al. (1996b), Vasquez et al. (2008) and Muhidinov et al. (2010), who worked with passion fruit, lemon, banana peel and apples, respectively, obtaining in all the studies an average value of 10.9% pectin. This is due to the pure galacturonic acid content not being measured in the present experiment.

Relationship between the degree of esterification and grams of dehydrated pectin

The relationship between the degree of esterification and the amount of dehydrated pectin indicated that the $T_1$ treatment, which was found to be the best extraction conditions, resulted in a 65.93% degree of esterification and 7.25% of dried pectin.

The $T_4$ and $T_5$ treatments, with a pH of 2.5, showed low degrees of esterification, 26.37 and 28.77%, respectively, which could be explained by the extraction method used, pectolytic enzymes and non-specific substances that may reduce the degree of esterification and reduce the purity of the pectin.

In terms of grams of dried pectin yield, the $T_4$ and $T_5$ treatments did not produce the highest values but showed a tendency to increase (not significantly), from 5.41 to 5.71%, which coincided with the results of Camejo et al. (1996a), Camejo et al. (1996b) and Vasquez et al. (2008), in which the degree of esterification decreased and the performance increased in relation to a low pH.

According to Vasquez et al. (2008) and Addosio et al. (2005), the increase in the pectin obtained, associated with a decrease in pH, is related to the extraction of different biomolecules present in the skin of the fruit, such as starch, hemicellulose and cellulose. In the present experiment, the process of hydrolysis at pH 3.0 and 2.5 could release many more components and mask the amount of pectin in comparison with that extracted in treatments $T_3$, $T_4$ and $T_5$.

Moreover, Pagani (1990) and Muhidinov et al. (2010) demonstrated that there is a breakdown of the water-soluble protopectin during hydrolysis, resulting in an increase in the extraction yield. Thus, it can be inferred that, in treatments $T_1$, $T_3$ and $T_5$ in which the apple juice was subjected to a 90 minute hydrolysis, there was an increased pectin molecule breakdown, generating an increased pectin yield.

Sensory evaluation of organoleptic attributes

Table 3 shows the values obtained from each of the treatments. In general, the judges gave different assessments regarding the sensory attributes of color, flavor, aroma and texture for all of the samples.

The color attribute varied from a light brown to a dark brown. According to the panelists, the samples from $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$ presented the lightest color (the same in each of them) but the color produced by the $T_4$ and $T_5$ (pH 2.5 for 90 min) treatments were significantly different; the latter treatment produced a darker pectin color (average 10.39 in the scale 1 to 13 explained in the
According to the panelists, the intensely acid taste of pectin was present in the samples of the $T_4$ (pH 2.5 for 60 min) and $T_5$ (pH 2.5 for 90 min) treatments, with averages of 11.19 and 11.52, respectively, which were significantly different from the $T_0$ and $T_1$ treatment samples. However, the flavors from the $T_2$ and $T_3$ treatments samples were perceived as acidic, similar to those from the $T_4$ treatment. The pH and time spent extracting the pectin samples influenced their identification by the panelists.

Regarding the aroma, significant differences between the $T_0$ (pH 3.5 for 60 min) and $T_3$ (pH 3.0 for 90 min) treatments, with averages of 11.19 and 11.52, respectively, which were significantly different from the $T_0$ and $T_1$ treatment samples. However, the flavors from the $T_2$ and $T_3$ treatments samples were perceived as acidic, similar to those from the $T_4$ treatment. The pH and time spent extracting the pectin samples influenced their identification by the panelists.

The evaluation of texture ranged from smooth to rough. According to the estimate by the panelists, there was a tendency to a rough texture, with similar values between 8.05 and 8.72 for treatments $T_0$, $T_1$, $T_2$, $T_3$ and $T_4$ and no significant differences among them. Only significant differences between the $T_2$ (pH 3.0 for 60 min) and $T_3$ (pH 2.5 for 90 min) treatments were found, with averages of 9.23 and 8.05, respectively.

According to the panelists, the results regarding the sensory attributes of the pectin samples, color, flavor and texture, averaged 8.34; these values are higher than those found by Vasquez et al. (2008) for banana pectin (4.20). However, in relation to the aroma perceived in the tested samples, the results were coincident with those described by the same authors, with an average of 4.0.

### Sensory evaluation of organoleptic attributes and acceptability

The results of the acceptability evaluation are given in Table 4. After the sensory evaluation, the panelists showed the same degree of preference for the pectin samples from the $T_0$ and $T_1$ treatments (statistically equal) and it can be inferred that these samples demonstrated the best acceptability, which was probably due to the conditions of the treatments. Within the range of 1 to 7, the $T_0$ and $T_1$ treatments were rated as 5.28 and 5.10, respectively, meaning that these samples were considered better than average by the judges.

### Table 3. Results of the sensory evaluation panel according to a non-structured chart.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Color</th>
<th>Flavor</th>
<th>Aroma</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_0$</td>
<td>6.35 ab</td>
<td>5.36 c</td>
<td>3.10 b</td>
<td>8.61 ab</td>
</tr>
<tr>
<td>$T_1$</td>
<td>7.69 ab</td>
<td>6.97 c</td>
<td>4.10 ab</td>
<td>8.57 ab</td>
</tr>
<tr>
<td>$T_2$</td>
<td>7.86 ab</td>
<td>9.48 b</td>
<td>4.10 ab</td>
<td>9.23 a</td>
</tr>
<tr>
<td>$T_3$</td>
<td>7.16 ab</td>
<td>9.55 b</td>
<td>4.37 a</td>
<td>8.72 ab</td>
</tr>
<tr>
<td>$T_4$</td>
<td>4.71 b</td>
<td>11.19 ab</td>
<td>4.71 a</td>
<td>8.72 ab</td>
</tr>
<tr>
<td>$T_5$</td>
<td>10.39 a</td>
<td>11.52 a</td>
<td>5.02 a</td>
<td>8.05 b</td>
</tr>
</tbody>
</table>

Means without a common letter are significantly different according to Tukey’s test at a significance level of 0.05. $T_0$: pH 3.5 for 60 min; $T_1$: pH 3.5 for 90 min; $T_2$: pH 3.0 for 60 min; $T_3$: pH 3.0 for 90 min; $T_4$: pH 2.5 for 60 min; $T_5$: pH 2.5 for 90 min.

### Table 4. Results of the sensory evaluation and acceptability according to a structured chart.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters of acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_0$: pH 3.5 for 60 min</td>
<td>5.28 a</td>
</tr>
<tr>
<td>$T_1$: pH 3.5 for 90 min</td>
<td>5.10 ab</td>
</tr>
<tr>
<td>$T_2$: pH 3.0 for 60 min</td>
<td>4.56 b</td>
</tr>
<tr>
<td>$T_3$: pH 3.0 for 90 min</td>
<td>4.56 b</td>
</tr>
<tr>
<td>$T_4$: pH 2.5 for 60 min</td>
<td>3.87 c</td>
</tr>
<tr>
<td>$T_5$: pH 2.5 for 90 min</td>
<td>3.59 c</td>
</tr>
</tbody>
</table>

Means without a common letter are significantly different according to Tukey’s test at a significance level of 0.05.

The only pectin samples with low acceptability, which were also statistically equal, are those from treatments $T_4$ (pH 2.5 for 60 min) and $T_5$ (pH 2.5 for 90 min), receiving values of 3.87 and 3.59, respectively, indicating that these samples were considered below average, probably due to the pH used. The $T_2$ (pH 3.0 for 60 min) and $T_3$ (pH 3.9 for 90 min) treatments showed a score of 4.56, which was average.

However, the intensity of sensory attributes perceived by the panelists did not allow the assessment of the best extraction procedure.
Regarding the analysis of acceptability, the $T_4$ and $T_5$ treatments were the pectin samples with greater appreciation by the panelists and were rated as the best. The samples that were less accepted were the samples from treatments $T_3$ and $T_5$, which were less than average.

**Resumen**

N. Loyola, P. Pavéz y S. Lillo. 2011. Extracción de pectina a partir de manzana (*Malus pumila*), cv. Pink Lady. Cien. Inv. Agr. 38(3): 425-434. El presente estudio tuvo como objetivo la extracción de pectina a partir de manzana (*Malus pumila*), cv. Pink Lady, con un promedio de madurez fisiológica de un 50% color rojo de cubrimiento, mediante técnicas químicas estandarizadas, para evaluar si esta variedad se caracteriza por un alto o bajo valor de metoxilo, mediante una hidrólisis ácida a diferentes tiempos y luego ser sometida a un análisis sensorial, evaluando las características organolépticas de la pectina. La materia prima fue obtenida del fundo Marengo de la localidad de los Niches, provincia de Curicó, Chile. El aislamiento del material péctico se realizó con ácido cítrico como agente de extracción, para ello se ensayaron tres condiciones de pH (2,5, 3,0 y 3,5, siendo este último el pH natural de la manzana) durante un tiempo de calentamiento de 60 y 90 minutos, sometidos a una temperatura constante de 90 ºC. Se midió el grado de esterificación en la pectina (DE), el cual es un atributo importante de ésta y luego se procedió a deshidratarla para evaluar sus atributos sensoriales como; color, sabor, aroma, textura y su aceptabilidad (escala 1-7). El tratamiento $T_1$ (pH: 3,5 por 90 min) presentó la mejor condición de extracción (4,47 g o 7,25%), siendo el tratamiento $T_0$ (pH: 3,5 por 60 min) el que otorgó la muestra de mejor calidad (68,27% DE), clasificándola como pectina HM (High Methoxy). La evaluación sensorial mostró que los tratamientos originan atributos sensoriales variables. Los panelistas mostraron igual grado de preferencias por las muestras de pectinas provenientes de los tratamientos $T_0$ y $T_1$ con nota 5,28 y 5,10, dentro de la escala 1 a 7.

**Palabras clave:** Análisis sensorial, esterificación, manzana, pectina.

**References**


