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Extraction of Pectin From Apple Pomace

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ABSTRACT

*As apple-processing units are now in expansion in Brazil, industrial by-products like pomace play an important role
in pectin manufacture. The objective of this article was to determine a pratical follow-up to the extraction of pectin
from apple pomace and to characterize it in a laboratory, on a small scale, aiming at establishing the optimum
conditions for acid extraction. The highest yields were obtained when [1]apple pomace was dried and ground to
obtain an apple flour to be used as raw material, [2] citric or nitric acids were used and [3] when the citric acid
concentration was 6.2 g/100 ml and the time of reaction was 153 minutes. The apple variety in itself was not
significant in pectin yield. The degree of esterification (DE = 68.84 %) of the product obtained, as well as its
physical looks, show the success of pectin extraction.*

Key words: Apple pomace, pectin, agricultural wastes, yield, extraction, Response Surface Methodology (RSM)

INTRODUCTION

Citrus albedo and apple pomace are rich in pectic
substances and important raw materials for pectin
production all around the world (Fox, 1984). There
is only one factory in Brazil producing citrus
pectin, in Limeira, State of São Paulo (Cpkelco,
2002) but none producing apple pectin. The
Brazilian apple production, however, quickly
spread in the Southern States of the Country in the
70's (Wosiacki, 2001) and in the 2002 / 2003 crop,
the apple production was estimated at 980,000
tons, with approximately 180,000 tons for
industrial processing. Nowadays, about 50,000
tons of wet pomace are produced in Brazil. A
possibility for large enterprises is the drying of this
wet raw material aiming at the production of apple
pectin (Endress, 2000). Apple pomace is produced
as a by-product of the juice factories and currently
it is either used for animal feeding or is disposed
of as an industrial waste. If apple pomace could be
used the production of pectin, around 2000 tons of
pectin could be produced each year.
Pectin is a family of complex variable
polysaccharides extracted from the primary cell
wall of higher plants. Chemically, pectin consists
of linear polymers of D-α-(1$\rightarrow$4) anhydro-
galacturonic acid. Part of the carboxyl groups of
the anhydro-galacturonic acid is esterified with
methanol (Wosiacki, 1977). Vauquelin stated its
chemical nature in 1790 and Braconnot showed
the characteristic of geleification and gave it the
name pectin (Berk, 1976). Pectin is widely used as
a functional ingredient in the food industry due to
its ability to form aqueous gels and has been used
in jams and jellies, fruit preparations, fruit drink

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concentrates, fruit juice, desserts and fermented dairy products. Commercial pectin is currently classified according to the degree of esterification (DE). There are three classifications of pectin: HM (high ester); LMC (low ester conventional) and LMA (low ester amidated) according to CPKelco (2002).

Pectin in apple pomace is mainly present in the form of protopectin, an acid soluble polysaccharide. The condition for the acid extraction of pectin is somewhat obscure in the literature and it is very difficult to visit processing units. There are complicated procedures in its study (Thibault et alli, 1991), although pectin has been under investigation since the start of 20th century (Kertesz, 1951). Some experimental research should be done in order to clarify this important industrial step so as to utilize apple pomace as the main raw material for the extraction of pectin. The objectives of this work were to extract pectin from apple pomace on a laboratory scale and to characterize it in order to observe the influence of some factors on the yield of pectin.

MATERIALS AND METHODS

Materials

Samples from nine different apple varieties (Belgolden, Catarina, Eva, Fred Hough, Fuji, Gala, MRC, Rainha and Sansa) were used as raw material in this work. They were processed together in order to obtain an apple pomace pool, according to industrial procedure. In the laboratory, all the fruits were first washed and ground in an electric grater. The crushed pulp was then pressed and the apple pomace dried, initially at room temperature and then at 50ºC, to a constant weight with air circulation. This dry apple pomace pool was then crushed and mixed and the product was called apple flour and it was used as the raw material for all the assays made concerning pectin extraction and characterization.

Three apple flour varieties were selected (Belgolden, Catarina and Eva) in order to establish the influence of apple variety on the yield of pectin extraction and the results were compared with a pomace pool of all the apple varieties.

Pectin extraction

Five replicates for each assay were made. Pectin was extracted under reflux in a condensation system at 97º C for 30 min (solute/solvent 1:50),

using water acidified with citric acid to pH 2.5, using apple flour (pool) as raw material. Next, the pool was classified with four metallic sieves (1,18 mm, 600 μm, 250 μm and 106 μm) in a shaker device in order to separate them in five portions with different particle sizes. Three batches, as well as crude dried pomace without crushing were used to observe the effect of particle size on pectin yield. The extraction of pectin was performed with apple pomace from selected varieties under equal conditions.

Pectin extraction was also performed under reflux at 97ºC for 30 min (solute/solvent 1:50), using water acidified with different acids, to investigate the effect of each kind at pH 2.5 and to ratify the use of citric acid. In this assay, the raw material selected was the apple flour pool with particles between 250 and 105 μm. The selected acids utilized for extraction were citric, phosphoric, hydrochloric, nitric, malic, tartaric and sulfuric acids.

Pectins extracted in each trial were all mixed and the degree of esterification was analyzed (Gee et al., 1959). The analysis of variance (ANOVA) was used to define the optimum conditions concerning the acid used for pectin extraction, the apple variety and the size of particles of the apple pomace.

Isolation of pectin

Hot acid extract was pressed in a cheese cloth bag and the concentrated "juice" was cooled to 4º C. The apple pectin was precipitated by alcohol-juice treatment 2:1 (v/v). The mixture of solvent and precipitate was stirred for ten minutes and then left to rest for one hour in order to allow pectin flotation. With this procedure the pectic substances remain at the surface of the alcohol/water mixture and thus it is easier to remove them in a quantitative way.

The floating pectin was filtered through cheesecloth, rinsed with 95º GL alcohol and then pressed. The pressed pectin was dried to constant weight at 55 ºC, cooled in a dessicator and the yield calculated on a dry weight basis (inicial weight of sample). The hard pectin cake was broken up, ground and sieved in order to obtain powdered pectin.

Experimental design

Response surface methodology was used to optimize pectin extraction with respect to time and

acid concentration; the temperature was maintained constant at 97ºC, the boiling point of water in the laboratory. A five-level two-factor fractional design was adopted. The response surface model equation is (1):

$$Y = b_o + b_1x_1 + b_2x_2 + b_{11}x_1{}^2 + b_{22}x_2{}^2 + b_{12}x_1x_2 \ (1) \qquad \textbf{(1)}$$

where Y is the response variable of pectin yield (%); b_1 and b_2 are regression coefficients for linear effects; b_{12} is the regression coefficient for interaction effects; b_{11} and b_{22} are regression coefficients for quadratic effects, and x_1 and x_2 are coded experimental levels of the variables. Statistica for Windows was used to match the quadratic equation with the experimental data.

Optimization

The apple flour suspensions were heated at 97ºC under reflux with a glass condensation device. The factors studied were citric acid concentration (CAC = 0.05-9.95g %) and heating time (HT = 10-210 min). Citric acid was always added to the flasks when the system reached the boiling point. The experiment was conducted using a five-level two-factor composite design with three assays at the central point. In order to maximize the pectin extraction, coded and real levels, based on previous experiments, were selected. Both factor variations can be seen in Table 1. The test runs were performed in a random order according to a Statistic for Windows tool.

Table 1 - Coded and real levels of variables

Variable	Coded level of variables				
	$-\sqrt{2}$	-1	0	+1	$+\sqrt{2}$
X₁: citric acid, g/100 ml	0.05	1.5	5	8.5	9.95
X₂ : time, min.	10	39.3	110	180.7	210
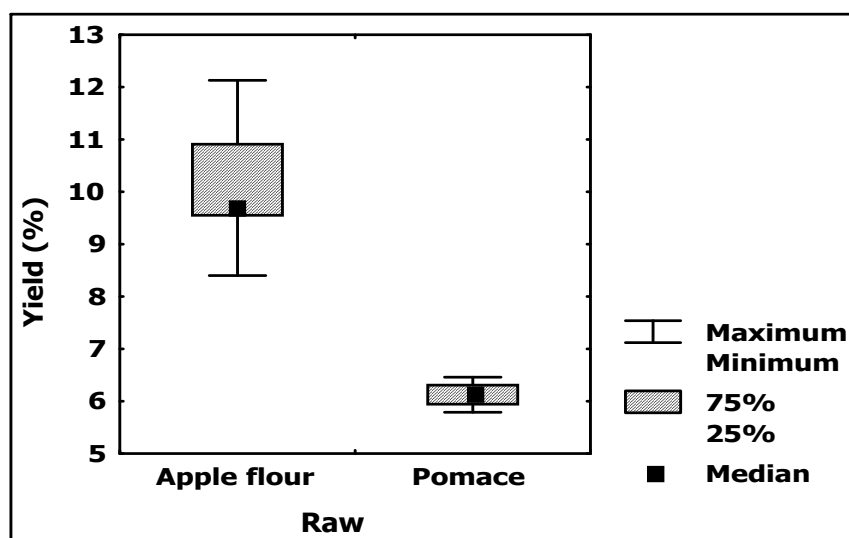
Figure 1 - Effect of particle size on the yield of extraction using apple flour and apple pomace as a pectin source.

RESULTS AND DISCUSSION

Effect of the size of the particles
According to the results (Fig. 1), the pectin yield was significantly higher with the use of flour as raw material (9.73 %); when the extraction was made from the pomace the yield was 6.13%. There are several explanations for this fact, but protopectin is more available in small particles than in large ones.

Three apple flour fractions were collected during the study of the particle size and used to confirm these results.

The flour fractions with particles larger than 600 µm showed lower pectin yield while when smaller particles were used the yield was higher (Fig. 2).

Effect of apple variety
Although some varieties do have a high concentration of soluble pectin in the apple juice, the pectin extracted from the pomace did not show a pronounced variation, although there was a statistical difference amongst the varieties. The blend of varieties results in a raw material more similar to industrial raw pomace constituted of various apple varieties in indeterminate proportions and several maturation stages (Fig. 3).

Effect of different acids on pectin extraction
Several acids can be utilized for the extraction of pectin. According to Kertesz (1951), the acids used for pectin extraction were the tartaric, malic, citric, lactic, acetic and phosphoric acids but there was a tendency to use the cheaper mineral acids, such as sulfuric, hydrochloric and nitric acids. Articles published recently recommend the use of hydrochloric (Kalapathy and Proctor, 2001; Hwang et al., 1998; Dinu, 2001) and nitric acids (Pagán et al., 2001).

According to Fig. 4, the lowest yields were obtained when phosphoric and malic acids were used. Although nitric acid showed the highest yield, the variation was very large. Citric acid had the highest average value (13.75%) and it is better than the other acid from an economic as well as from an environmental point of view. The analysis of variance indicated statistical significance in the yield.
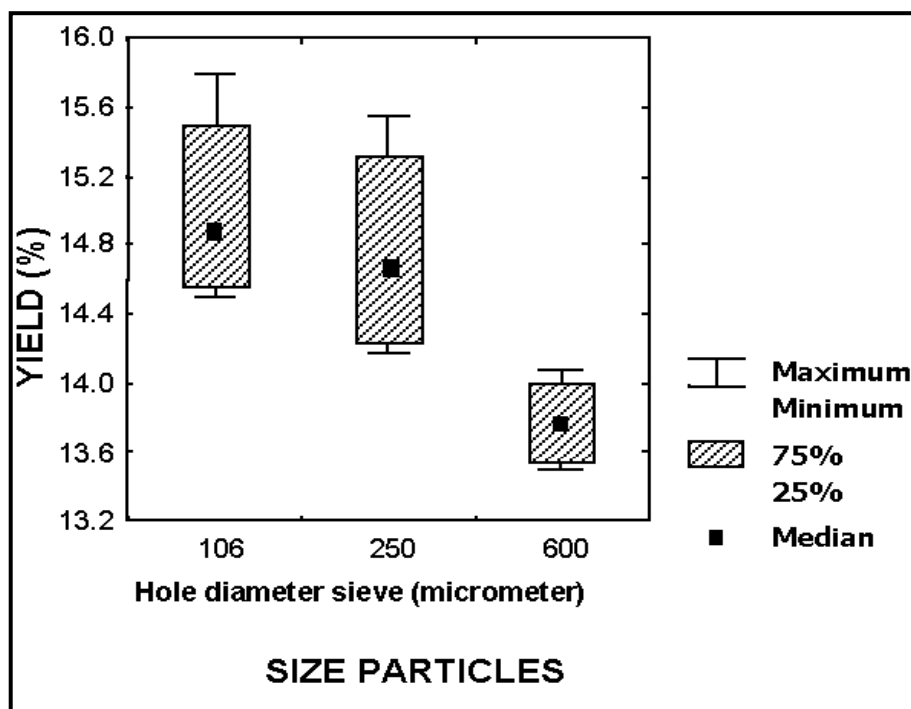

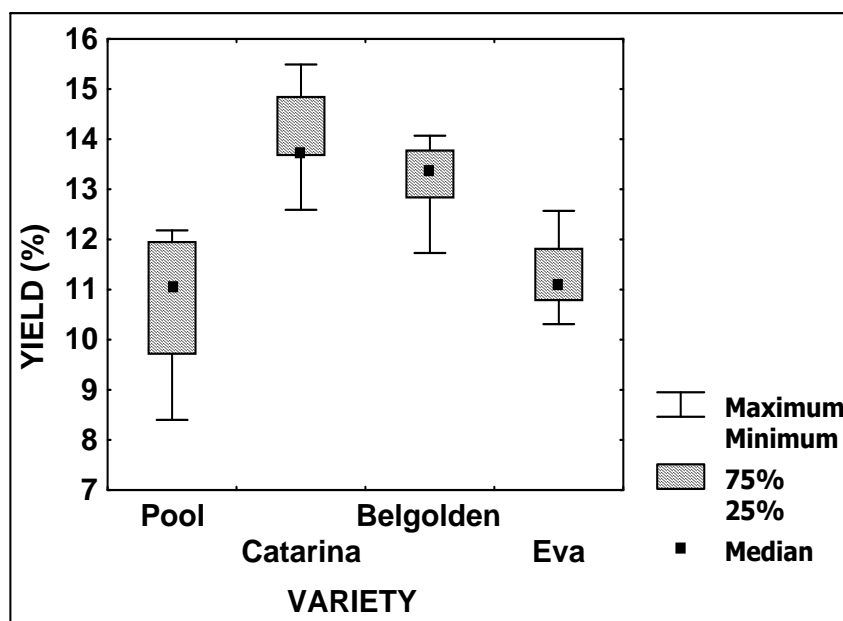**Figure 2 -** Effect of particle size on the pectin extraction yield.

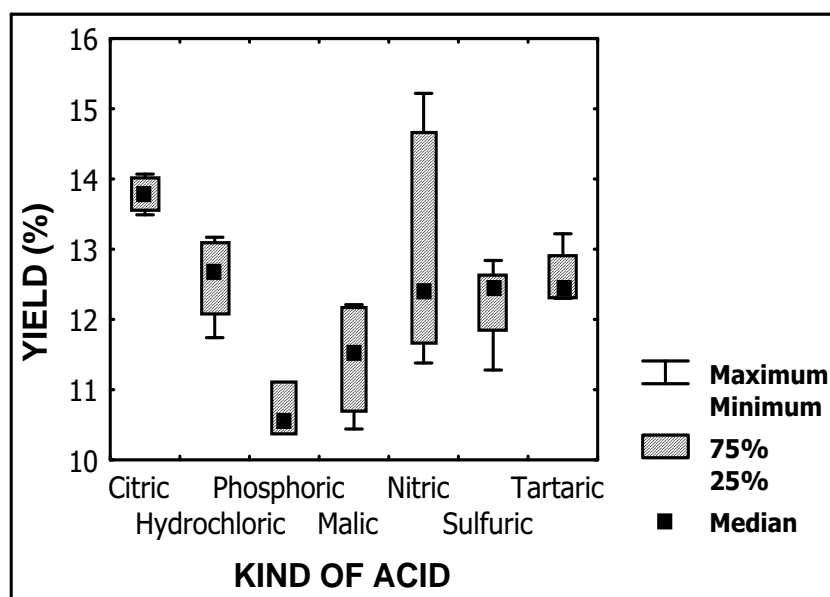**Figure 3 -** Effect of apple variety on the pectin extraction yield.

Figure 4 - Effect of the kind of acid on the pectin extraction yield.

Optimization of yield

Table 2 shows the experimental design and its results. The average results of the central coordinates were 20 ± 0.86, which represented a low variation coefficient of 4.8%.

The regression analysis showed that 93.55 % of the variations was explained by the model. The predictive equation explains a surface with a maximum point with coordinates X1=152.85 minutes and X2= 6.2 g % of citric acid with a theoretical maximum yield of 17.82 g % of pectin on a dry basis (Equation 2).

$$z = 19.9957 + 0.8819x - 2.4457x^2 + 1.3865y - 1.5613y^2 + 1.4215xy + 0 \quad (2)$$

The results indicated that the yield of pectin was dependent on the linear terms, on the quadratic terms and also on the interactions of both variables. Table 3 illustrates the coefficients of the regression model for the determination of pectin.

Both linear and quadratic effects showed significance in accordance with the analysis of variance. The experimental design indicated the formation of a surface where the pectin extraction yield was optimum, between 18 and 20%. Fig. 5 illustrates this surface in three dimensions, clearly

showing a critical point of high yield with an equation statistically significant according to ANOVA, since F_{cal} (6.005665) $>F_{crt}$ (4.256492).
The degree of esterification of the apple pool pectin was 68.84% on average, as could be expected from mature apples.

Table 2 - Experimental design and pectin extraction yield.

| Run | Level | | | | Yield (g%) |
| | Coded | | Real | | |
	Citric acid (g%)	Time (min.)	Citric acid (g%)	Time (min.)	
1	-1	-1	1.5	39.3	15.59
2	-1	1	1.5	180.7	15.88
3	1	-1	8.5	39.3	13.30
4	1	1	8.5	180.7	19.27
5	-√2	0	0.05	110	12.98
6	√2	0	9.95	110	17.18
7	0	-√2	5	10	15.14
8	0	√2	5	210	18.56
9	0	0	5	110	20.92
10	0	0	5	110	19.23
11	0	0	5	110	19.83

Table 3 - Analysis of variance of regression model of experimental design.

| Factor	Regression Coefficient	Standard Error	T(s)	P	-95% Cnf.Limt	+95% Cnf.Limt
Mean/ Interaction	19.9956	0.5580	35.8305	3.19E-07	18.5611	21.4302
(1) Citric acid (L)	1.7639	0.6835	2.5805	0.0493	0.0068	3.5209
(2) Citric acid (Q)	-4.8913	0.8136	-6.0114	0.0018	-6.9829	-2.7997
(3) Time (L)	2.7730	0.6835	4.0568	0.0097	1.0159	4.5301
(4)Time (Q)	-3.1225	0.8136	-3.8376	0.0121	-5.2142	-1.0309
1L * 2L	2.8430	0.9665	2.9412	0.0322	0.3583	5.3277

L= linear effects
Q= quadratic effects

CONCLUSIONS

When the effects of particle size were studied it was found that the highest average yields of around 14% were obtained when the particle was larger than 106 μm and smaller than 250μm.
Extraction of pectin using pomace as raw material produced a lower pectin yield than when apple flour was used. This result indicated that it was necessary to produce apple flour as an intermediary step in the acid extraction of pectin from pomace. Pectin extraction from different

varieties of apple pomace did not show any significant effect on pectin yield as compared with a pool of apple samples. Citric acid and nitric acid showed the highest yield among the organic and mineral acids tested.
The coordinates of the stationary point were found to correspond to 6.2 g/100 ml of citric acid with a reaction time around 150 min. The apple pool pectin showed a degree of esterification of approximately 68.84%.
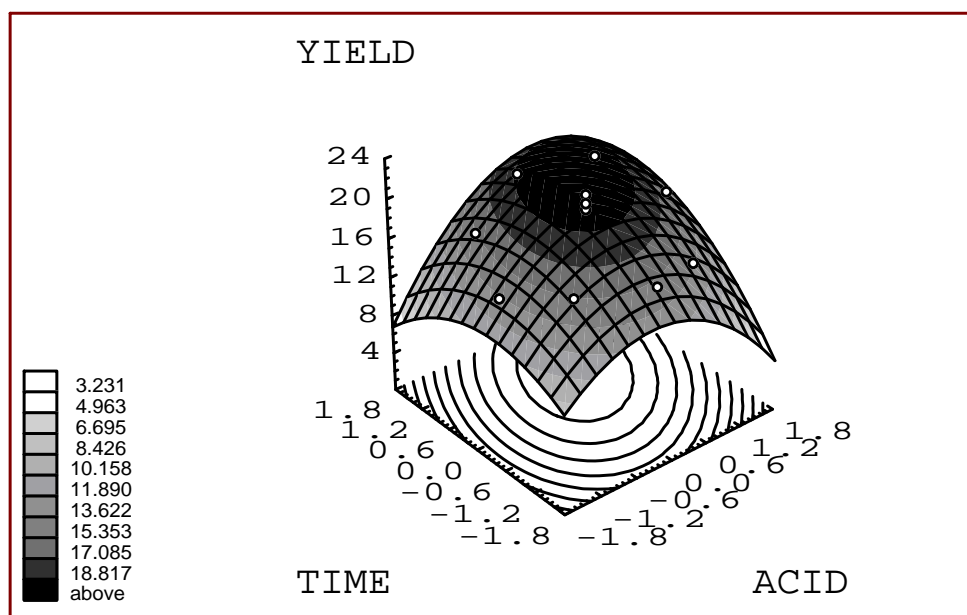
Figure 5 - 3-D dimension response surface of experimental design (five-level two-factor composite) on the pectin extraction yield.

RESUMO

No aproveitamento de resíduos das indústrias processadoras de maçã, em expansão no Brasil, a pectina alimentícia surge como importante subproduto. Os objetivos deste trabalho foram os seguintes: extração e caracterização da pectina de bagaço de maçã em bancada, em que as condições para maior rendimento de obtenção em relação ao tipo de ácido, ao tamanho das partículas e à variedade de maçã foram estabelecidas. Objetivou-se também a otimização da produção, levando-se em conta o tempo e a concentração do ácido cítrico. Foram considerados métodos de extração mais eficientes quanto ao rendimento: [1] acidificação com ácido cítrico ou ácido nítrico, [2] cominuição do bagaço, usando a farinha como matéria-prima, com partículas retidas no tamis de 106 μm, [3] concentração de ácido de 6,2 g% e tempo de extração de 153 minutos. A variedade da maçã não interferiu significativamente no rendimento. A alta metoxilação (DE= 68,84%) das pectinas produzidas em bancada confirma o grau de maturidade das matérias-primas usadas nos experimentos.

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