APPLE PULP ENZYME TREATMENT WITH ULTRAZYM®AFP-L AND PANZYM®YIELDMASH

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The present study aimed to verify the influence of the enzymatic preparations Ultrazym®AFP-L and Panzym®YieldMASH over the yield and chemical quality of apple juice. The process was optimised according to two parameters: temperature and enzyme concentration in a one hour-long run. The results of physical and chemical analysis were further submitted to multivariate statistical procedure aimed at pattern recognition. The exploratory and classificatory modeling discriminated the samples with 100 % correlation. The results of the experimental factorial design pointed to enzyme concentration as being the main factor that increased the yield. The concomitant observation of data using Principal Component Analisys (PCA) exploratory tools showed the tendency to separate the control samples according to higher content of phenolic compounds, glucose, pH and colour; the Ultrazym® AFP-L samples according to total acidity; and the Panzym®YieldMASH samples according to higher sugar content. It was concluded that the utilization of industrial enzymes in apple processing increases the yield of juice and decreases the amount of pomace.

KEY-WORDS: ENZYME PREPARATION; EMPIRICAL MODELING; MULTIVARIATE ANALYSIS; ULTRAZYM® AFP-L; PANZYM®YIELDMASH; APPLE.

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1 INTRODUCTION

Brazilian apple production is approximately 1.500 million ton per year (FAO, 2012). Consumers have high expectations regarding quality (size, appearance, shape, etc.) when it comes to fresh fruit, therefore those fruits that do not meet these standards are send for industrial processing (PROTZEK *et al.*, 1999; WOSIACKI, PHOLMAN & NOGUEIRA, 2004; FERTONANI *et al.*, 2006).

Conventional apple juice processing is based on the mechanical extraction of total soluble solids from physically disrupted cells in milled fruit and it utilises a pectinolytic enzyme treatment in fining operations, which is designed to produce a juice with good appearance (RIBEIRO *et al.*, 2010). The process requires large presses with optimised performance in order to reach 75 % yield, leading to a good quality product after fining and the release of pomace as the main residue (ISSENHUT & SCHNEIDER, 2008). Due to its composition, pomace must be immediately dried and the resulting powder, with around 12 g/100 g of moisture consists of a biomass rich in sugars (40 g/100 g) and total fibre (44 g/100 g); a suitable raw material for further processes on the recovery of functional compounds (SANTOS *et al.*, 2005) or even for alcoholic fermentation (NOGUEIRA *et al.*, 2005).

The use of enzyme in several stages of apple juice processing is an old industrial praxis (URLAUB, 1996) and commercially available pectinases have many different pectinolytic activities (GRASSIN *et al.*, 2005). Their use in the mashing stage has been proposed as a way to increase apple juice yield by breaking some chemical linkages in cell wall polysaccharides, which allows the fluid inside the cell to be easily removed (DEMIR *et al.*, 2001). Enzymes used are amylase, protease, pectinase, hemicellulase and cellulase but, according to German regulations, the last two are only allowed as side effects (STUTZ, 1996). If cellobiose is detected in apple juice this attests that it was, in some way, adulterated (HOFFSOMMER, 2006) and the idea of total liquefaction is no longer appropriate because the seeds and the epidermis, also components of fruit, are not involved (STUTZ, 1996). Such a change in processing led to a modification in the type of equipment; decanters or centrifuges are now used instead of traditional presses (STUTZ, 1996). The amount and quality of the pomace are also altered when depolymerising enzymes are used in the mashing stage (URLAUB, 1996) and yields reach a level of 80-85 % (ISSENHUT & SCHNEIDER, 2008).

Total liquefaction is not necessary for total fluid extraction from physically broken fruit cells, but the enzyme industry has provided alternatives that allow the preparation of cocktails of enzymatic activities according to customers' specifications, either with or without cellulase (STUTZ, 1996; MEHRLÄNDER *et al*., 2002). This technical knowledge enabled the production of enzymes in industrial units, which resulted in optimised mash enzymation (OME) and the setting up of mixtures known as advanced fruit processing (AFP). These techniques created the possibility of breaking down pectin, hemicellulose and cellulose in conditions that improve each reaction, according to the local legislation. This represented a new way of meeting the needs of the international market, including the European Union.

The screening of enzyme source and a proper purifying procedure lead to a very active polygalacturonase, which provides better results than if crude or impure sources are employed. However, this target is of low magnitude (+1 % TSS) but has a good impact, as the last generation pectinolytic enzyme is yet not available.

The effect of pectinases (NOGUEIRA *et al.*, 2005) and cellulases (WILL, BAUCKHAGE & DIETRICH, 2000), which release compounds such as d-galacturonic acid and neutral sugars (GRASSIN & FAUQUEMBERGUE, 1996) linked to pectin substances, specifically glucose to cellulose (GOMIS *et al.*, 2004), along with the main total reducing sugar, may increase the sugar fraction to higher levels. Compared to conventional juice processing, the enzymatic treatment of the pulp has many advantages, but apart from the arguments regarding types of equipment (presses vs. centrifuges) the presence of cellulase means that it is rarely used, especially in the European Union. For Will *et al.* (2003) and Jaroslaw *et al.* (2009), the disadvantages of the process are: the lack of sensorial quality; the increase in polyphenol concentration; the promotion of browning reactions and

the increase in costs. However, the product has a higher nutritional appeal due to the high levels of soluble compounds and polyphenols, as well as partially degraded fibre and flavonoids (WILL, BAUCKHAGE & DIETRICH, 2000).

Because of the magnitude and economic power of the apple juice industry, a new generation of smash was released on the industrial market, namely, Panzym®YieldMASH. This enzyme is suitable for the first smash, improving the yield and favouring stages in the downstream process, as well as being suitable for all kinds of raw materials (ISSENHUT & SCHNEIDER, 2008).

The present research studied apple juice processing with Ultrazym®AFP-L, containing cellulases and polygalacturonase, and with Panzym®YieldMASH, containing only last generation polygalacturonase.

2 MATERIAL AND METHODS

2.1 MATERIAL

A single sample batch (40 kg) of Fuji apples, 2009/2010 crop, were used, as well as specific enzyme preparations from Novozymes, Ultrazym®AFP-L (cellulases+polygalacturonase) provided by the Latin American representative of LNF, Bento Gonçalves (RS, Brazil) and Panzym®YieldMASH (polygalacturonase) from Begerow (Germany), which were maintained under refrigeration (10 ºC).

2.2 METHODS

2.2.1 Apple juice extraction by mechanical pressing

Milled apples were pressed to extract the premium juice and then Pectinex® Novozymes was added (3 mL/hL) to the juice, which was subsequently clarified at room temperature. This was then filtered in paper at atmospheric pressure. The juice was treated with gelatin (3 g/hL) and bentonite (40 g/hL) in order to remove undesirable chemical compounds and to enhance the appearance of the final product (NOGUEIRA *et al.*, 2003).

2.2.2 Apple juice extraction by centrifugation

The apples were selected, washed, weighted and ground until they became a pulpy mash. One hundred grams of this mash was poured into a 250 mL Erlenmeyer flask, and after reaching the proper temperature the enzymes were added directly to it and left for one hour in the shaker (150 rpm). The juice was then extracted in a centrifuge at 10.000 rpm *g* and submitted to the same treatment with gelatin and bentonite of conventional processing.

2.2.3 Physicochemical analysis

The pH was determined using a digital potentiometer (TECNAL TEC-5) (IAL, 2008). The total acid was evaluated by titration with NaOH 0.1 N and expressed as malic acid (MAL) in g/100 mL (IAL, 2008). Total soluble solids (TSS) were determined by refractometry and expressed in degrees Brix, corrected to 20 °C. The levels of reducing sugars (RS) and total reducing sugars (TRS) were determined by the colorimetric technique adapted by Nelson (1944), and the gravimetric method of Somogyi (1945), directly, and after sucrose hydrolysis with HCl (0.1 N) at 65 °C, 5 min, respectively. The D–glucose was determined by the enzymatic technique with glucose oxidase (GOD). Total phenolic compounds (TPC) were determined by the Folin–Ciocalteau colorimetric method, as described by Singleton, Orthofer and Ventos (1999). The intensity of colour was obtained by the sum of the absorbance at 440 and 520 ηm, which corresponded to the polyphenol and anthocyanin pigments, respectively, using UV/VIS equipment (UV mini 1240, Shimadzu).

The optimisation of the apple juice extraction using the enzyme preparations Ultrazym®AFP-L and Panzym®YieldMASH was evaluated by empirical modelling and response surface analyses from the initial factorial design. Mathematical treatment was performed using the Excel® programme. The response variable was the juice yield, in g/100 g. The samples of mashing enzymes were used in a separate experiment, aiming to determine the effect caused in gravimetric yield and in physicochemical attributes (Table 1).

TABLE 1 - THE EXPERIMENTAL DESIGN USED AND THE INPUT VARIABLES

T (°C)	Concentration of enzyme (µL/g)				Enzyme
20	0.00	1.25	6.25	11.65	Ultrazym®AFP-L
20	0.00	1.25	6.25	11.65	Panzym®YieldMASH
25	0.00	1.25	6.25	11.65	Panzym®YieldMASH
30	0.00	1.25	6.25	11.65	Panzym®YieldMASH
35	0.00	1.25	6.25	11.65	Ultrazym®AFP-L
50	0.00	1.25	6.25	11.65	Ultrazym®AFP-L

2.2.4 Multivariate and Statistical Analysis

Data were presented as mean ± standard deviation. The results of the experiments were analysed by one-way ANOVA, to a 95 % confidence level, and the differences were qualified using Tukey's differential method at 5 % probability, using STATISTICA 7.0 software (STATSOFT, 2009).

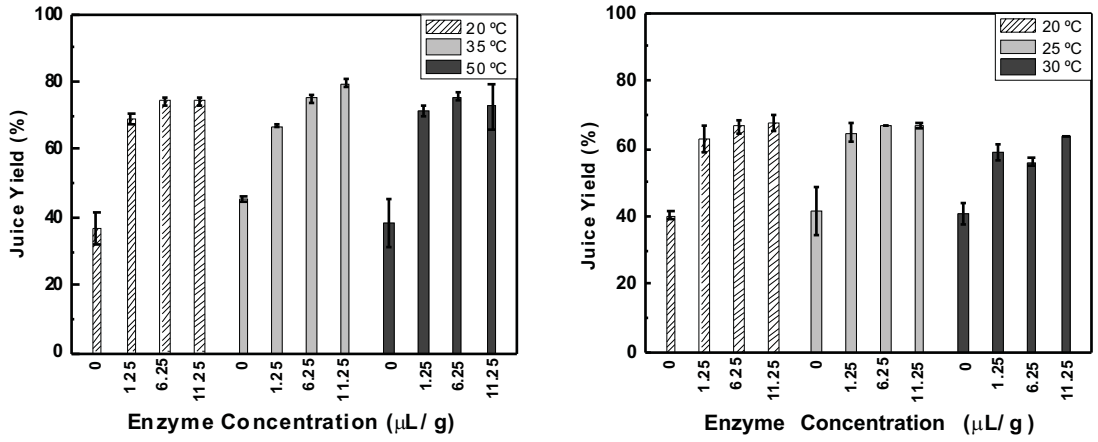
Principal component analysis (PCA) was used to explore the physicochemical data with a matrix of eight variables (output) from each of the juice samples that were analysed. The matrix was divided into eight classes according to the concentration and type of enzyme preparation. The analysis was performed using the Pirouette 4.0 programme (INFOMETRIX, 2008) and auto scale pre-processing. Soft independent modeling of class analogy (SIMCA) was also applied to build classification models according to the preparation method and enzymatic preparation. It was also performed on Pirouette 4.0 (INFOMETRIX, 2008).

3 RESULTS AND DISCUSSION

3.1 YIELD OF JUICE EXTRACTION

The apple pulp was submitted to a 1 hour treatment at several temperatures but without any enzymes being used, as a control run. The yield of juice extraction had an average value of 41.16 ± 2.04 g/100 mL and a fairly low variation coefficient (4.96 %) as compared to that of the variable temperature (38.00 %). This indicates either a minimal or non-existent effect on the output variable, i.e. the yield (Figure 1). It must be stated that as the apple variety was the same in both runs, but not from the same batch, the results are considered and expressed in terms of tendency and congruency. Any correlation between the enzymes and their activity must be carefully stated since the preparations that were used were different in many aspects, such as microbial source and fermentation process.

The addition of enzymes made it possible to achieve higher yields of apple juice, up to 32 % (Ultrazym®AFP-L) and 24 % (Panzym®YieldMASH), compared to the controls (Figure 1). The two preparations had intrinsic differences with relation to the kind of enzyme included; Ultrazym®AFP-L has high hydrolyses activity due to the action of pectinases and cellulases, while Panzym®YieldMASH contains new generation polygalacturonases (ISSENHUT & SCHNEIDER, 2008). However, both enzyme preparations showed similar behaviour regarding temperature because the yield profile was very closely related, including the trials with the highest temperature, in which there was a decrease in measured yields.



(A) **(B)**

FIGURE 1 - EXTRACTED JUICE YIELD BY CENTRIFUGAL FORCE (800 *g*) OF PULP USING (A) ULTRAZYM®AFP-L AND (B) PANZYM®YIELDMASH

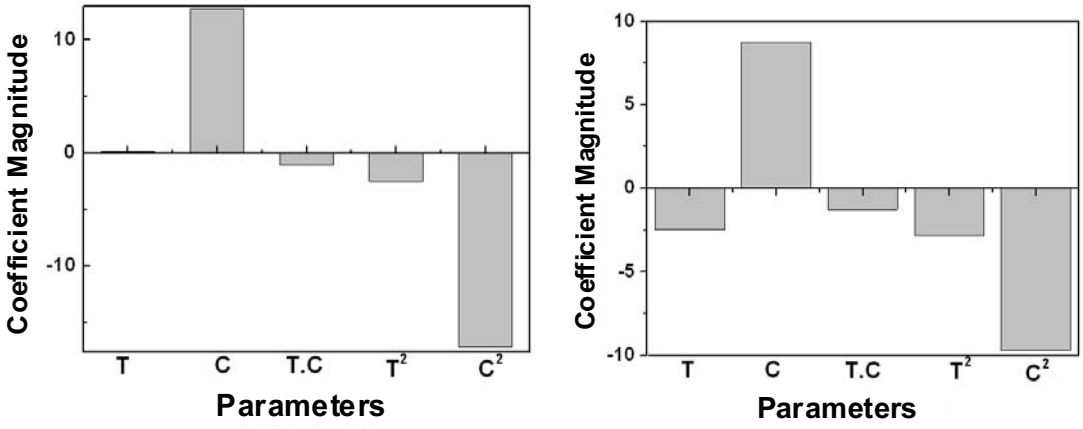
3.2 OPTIMISATION OF APPLE JUICE EXTRACTION

The factorial design that was followed to optimise the production of juice by the enzyme preparations Ultrazym®AFP-L and Panzym®YieldMASH points to linear and quadratic effects of the temperature and enzyme concentration and also their interactive effects, which were achieved by multiple linear regressions and also provided a regression equation. Figure 2 shows the representation of the regression coefficients of the variables. It was observed that the concentration of the enzymatic preparation Ultrazym®AFP-L was the most important parameter in relation to the juice yield, as shown by its coefficient. The influence of temperature over the response was less significant when compared with the interaction parameters and the quadratic models. On the other hand, for the Panzym®YieldMASH preparation, both temperature and concentration had a substantial effect, but the latter was the most significant. Concentration was also directly related to juice yield, whereas temperature presented an inverse relation. The influence of other parameters was smaller and inversely proportional.

The results showed that the Ultrazym®AFP-L sample produced higher yields of juice than the Panzym®YieldMASH sample. Analysis of factorial design indicated that the concentration of these enzymes is a more important factor than the temperature, as was observed by Oliveira *et al.* (2006).

Figure 3 shows the response surface for the enzymatic preparation and the studied variables. It shows the juice yield as a function of concentration and temperature. The representations of that figure were made by statistically significant variables for the responses. There was a strong negative quadratic relationship, mainly for the highest concentration of enzymatic preparation concentrate

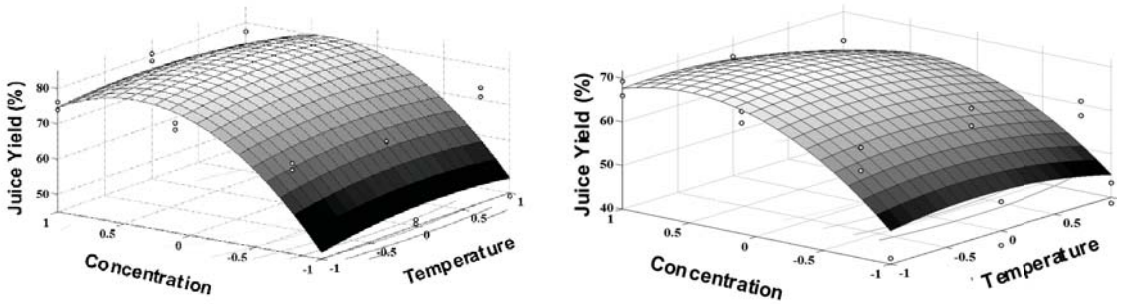
(Ultrazym®AFP-L and Panzym®YieldMASH), which indicated a normal distribution of data.



(A) Ultrazym®AFP-L **(B) Panzym®YieldMASH**

FIGURE 2 - COEFFICIENT REPRESENTATION OF THE CONTINUOUS VARIABLES OF OPTIMISED APPLE JUICE EXTRACTION BY CENTRIFUGATION

Figure 3(A) presents R^2 = 0.89 and it is possible to determine the optimum conditions by derivation (35 °C/7.74 μL/g of enzyme), while for figure 3(B) R^2 = 0.81 was observed, with optimum conditions (23 °C/8.32 μL/g of enzyme). The values of optimised concentration were quite similar and there was a deviation of 7.5 %. However, the difference between optimised temperatures was considerable, being 12 ºC higher for Ultrazym®AFP-L than for Panzym®YieldMASH.



(A) Ultrazym®AFP-L (B) Panzym®YieldMASH

FIGURE 3 - SURFACE RESPONSE GRAPHIC ILLUSTRATING THE TREND OF JUICE YIELD AS A FUNCTION OF TEMPERATURE AND ENZYME CONCENTRATION

3.3 PHYSICOCHEMICAL CHARACTERISATION

The results of the physicochemical analysis of the control and the enzymatic trials with Ultrazym®AFP are shown in Table 2.

A variation of 19.88 % was observed between the TRS values, ranging from 11.61 ±0.48 g/100 mL to 14.49 ±0.44 g/100 mL. This variation was in accordance with the values determined by Oliveira *et al.* (2006) for the application of the same enzymatic preparation in juice:

13.76 ±1.60 g/100 mL. For RS values, there was a correlated variation of 38.63 %, with values ranging from 8.61 ±0.47 g/100 mL to 14.03 ±0.06 g/100 mL. The average values determined by Fertonani *et al.* (2006) for Fuji apple clarified juice were 11.42 ±0.50 g/100 mL, and the values determined by Carvalho *et al.* (2010) were 11.33 ±0.39 g/100 mL. The Brix levels showed a variation between the treatments of 10.34 %, with values ranging from 13.00 °Brix to 14.00 °Brix. Rizzon, Bernardi and Miele (2005) determined °Brix values for Fuji apples in the order of 14.0, with a coefficient of variation of 7.7 %. During each temperature treatment, the addition of different concentrations of enzymes was expected to result in a sharp increase in Brix levels because polysaccharides, oligosaccharides, monosaccharides and polyphenols are the substances mainly responsible for the Brix increase during the enzymatic action (WILL, BAUCKHAGE & DIETRICH, 2000; WILL *et al.*, 2003). The levels of D-glucose showed a variation between treatments of 26.60 %, with levels from 3.09 ±0.11 g/100 mL to 4.21 ±0.04 g/100 mL. The values determined by Oliveira *et al.* (2006) for the application of the enzyme preparation Ultrazym® AFP-L were in the order of 2.31 ±0.04 g/100 mL.

TPC values varied by 33.72 %, with levels from 61.11 ±11.90 mg/L to 243.06 ±11.47 mg/L. Nogueira *et al.* (2003) found values for TPC in clarified juices in the order of 256 mg/L and observed that results were reduced by 56 % in the levels of catechins for Fuji apples compared to raw juice, after the depectinisation process.

Lightening in the colour of juices, with an increased concentration of enzymes, was observed during both treatments, as pectinases hydrolysis pectic substances from the medium lamella of cell wall and the resulting products may be removed with an impact in the final product characteristics, such as colour (RIBEIRO *et al.*, 2010).

The acidity variation ranged from 0.19 ±0.00 g/100 mL to 0.41 ±0.00 g/100 mL, representing a difference between the control tests (no enzyme treatment) and the enzymatic treated samples of up to 53.65 %. According to Oliveira *et al.* (2006) and Pool (1993), this high difference between samples is related to the liberation of D-galacturonic acid from the hydrolysis of the pectin chain. Will, Bauckhage and Dietrich (2000) also reported that D-galacturonic acid is responsible for an increase in total acidity. In the present study it was also possible to observe the difference between the enzyme treated samples; higher concentrations of enzymes resulted in higher levels of acidity, with the enzyme concentration influencing the liberation of D-galacturonic acid.

The results for Panzym®YieldMASH are shown in Table 3. The TRS values varied by 23.22 % between treatments, with levels ranging from 13.16 ±0.38 g/100 mL to 17.14 ±0.18 g/100 mL. The RS values ranged from 12.20 ±0.33 g/100 mL to 15.36 ±0.38 g/100 mL, with a variation between the results of 20.57 %. For the enzyme preparation, the levels of Brix showed values between 13.50 °Brix and 15.75 °Brix, with a variation of 14.28 %. The results for D-glucose showed a variation of 16.37 %, with the results ranging from 2.86 ±0.05 g/100 mL to 3.42 ±0.15 g/100 mL.

The TPC results showed a variation of 36.31 %, with values between 184.90 ±7.19 mg/L and 290.32 ±14.05 mg/L. A reduction in the TPC values was verified when the enzyme preparation was applied. According to Oszmianski and Wojdylo (2006), enzyme treatment affects the phenolic composition, resulting in phenolic oxidation and transforming quinines, which can polymerise and create coloured products in the juice. The clarification process removes these products and lightens the colour of the juice while enzyme concentration increases (OSZMIANSKI & WOJDYLO, 2006). Oszmianski, Wojdylo and Kolniak (2009) applied the same enzyme preparation in the mashing process to apple juice extraction. The preparation was reduced in polymeric procyanidins, compared with the control tests, which directly affected the total quantity of polyphenols. Differences were also observed between the control test and the samples with enzymes, with values of 715.3 mg/L and 649.0 mg/L, respectively.

Acidity levels showed an increase with the change of enzyme concentration in each treatment, with a variation of 53.57 % between samples and values ranging from 0.13 ±0.00 g/100 mL to 0.28 ±0.02 g/100 mL.

TABLE 2 - INFLUENCE OF TEMPERATURE AND ULTRAZYM®AFP-L ENZYME CONCENTRATION ON SOME QUALITY ATTRIBUTES OF APPLE JUICE

T (ºC)	C (µL/g)	TRS g/100 mL	RS g/100 mL	*TSS (°Brix)	D-Glucose g/100 mL	TPC mg/L	*pH	*Colour	Acid g/100 mL
20	0.00	13.30±0.45^{abc}	11.68±1.17^{bc}	14.00	3.40±0.28^{bcd}	243.06±11.47^{a}	4.28	2.56	0.21±0.01^{d}
	1.25	14.15±0.70^{a}	11.66±0.28^{bc}	14.50	3.39±0.14^{bcd}	215.63±10.51^{ab}	3.71	1.00	0.33±0.00^{b}
	6.25	11.56±0.61^{d}	12.31±1.74^{abc}	13.50	3.58±0.55^{abcd}	202.78±4.36^{bc}	3.54	0.58	0.39±0.00^{a}
	11.25	12.60±0.14^{bcd}	11.17±0.33^{bcd}	14.50	3.52±0.53^{abcd}	201.39±8.55^{bcd}	3.58	0.61	0.39±0.00^{a}
35	0.00	13.97±0.45^{ab}	11.80±0.17^{bc}	14.25	3.91±0.04^{abc}	211.46±9.85^{ab}	4.21	1.47	0.19±0.00^{d}
	1.25	14.16±0.11^{a}	12.10±0.39^{abc}	14.00	3.90±0.07^{abc}	220.14±10.65^{ab}	3.77	1.01	0.30±0.00^{c}
	6.25	14.23±0.59^{a}	12.83±0.25^{ab}	14.00	4.03±0.04^{ab}	194.10±17.05^{bcde}	3.55	0.57	0.39±0.01^{a}
	11.25	14.49±0.44^{a}	14.03±0.06^{a}	14.50	4.21±0.04^{a}	214.24±6.36^{ab}	3.53	0.56	0.41±0.00^{a}
50	0.00	11.72±0.67^{d}	8.61±0.47^{e}	13.00	3.22±0.10^{cd}	167.01±7.74^{de}	4.20	1.06	0.21±0.01^{d}
	1.25	11.61±0.48^{d}	9.41±0.09^{de}	13.50	3.53±0.08^{abcd}	161.11±11.90^{e}	3.72	0.67	0.31±0.01^{bc}
	6.25	12.24±0.33^{cd}	10.63±0.44^{cd}	13.75	3.09±0.11^{d}	217.01±21.53^{ab}	3.60	0.62	0.30±0.00^{c}
	11.25	12.40±0.54^{cd}	11.05±0.50^{bcd}	13.75	3.22±0.10^{cd}	173.96±9.54^{cde}	3.58	0.55	0.32±0.00^{bc}

Note: T = temperature; C = concentration of enzyme; TRS = total reducing sugars; RS = reducing sugars; TSS = total soluble solids; TPC = total phenolic compounds. Data expressed as mean ± standard deviation. Values marked with the same letter are not significantly different ($p<0.05$).

TABLE 3 - INFLUENCE OF TEMPERATURE AND PANZYM®YIELDMASH ENZYME CONCENTRATION ON SOME QUALITY ATTRIBUTES OF APPLE JUICE

T (ºC)	C (µL/g)	TRS g/100 mL	RS g/100 mL	*TSS (°Brix)	D-Glucose g/100 mL	TPC mg/L	*pH	*Colour	Acid g/100 mL
20	0.00	15.67±0.68^{abc}	15.36±0.38^{a}	14.75	3.00±0.05^{bc}	290.32±14.05^{a}	4.26	1.46	0.15±0.00^{f}
	1.25	15.83±0.61^{ab}	14.84±0.67^{ab}	15.25	3.27±0.11^{ab}	262.37±16.70^{ab}	3.67	1.28	0.22±0.01^{de}
	6.25	14.99±0.44^{bcd}	14.70±0.42^{ab}	15.25	3.12±0.06^{bc}	233.69±7.76^{bc}	3.52	1.18	0.26±0.00^{ab}
	11.25	13.99±0.45^{cde}	13.69±0.36^{bcd}	15.50	3.42±0.11^{a}	212.90±4.11^{cd}	3.50	1.08	0.28±0.02^{a}
25	0.00	13.84±0.72^{de}	13.11±0.67^{cde}	14.00	2.86±0.05^{c}	205.30±4.68^{cd}	4.29	1.08	0.13±0.00^{f}
	1.25	14.85±0.74^{bcde}	13.18±0.82^{cde}	14.00	3.11±0.05^{bc}	199.62±22.47^{d}	3.59	0.82	0.21±0.00^{e}
	6.25	16.94±0.11^{a}	14.78±0.20^{ab}	13.50	3.03±0.10^{bc}	186.36±10.53^{d}	3.57	0.76	0.23±0.00^{cde}
	11.25	13.67±0.77^{de}	12.20±0.33^{e}	13.50	2.94±0.11^{c}	202.65±9.11^{cd}	3.55	0.79	0.24±0.00^{bcd}
30	0.00	17.14±0.18^{a}	14.30±0.50^{abc}	14.75	3.12±0.05^{bc}	201.10±2.77^{d}	4.35	1.89	0.13±0.00^{f}
	1.25	13.16±0.38^{e}	12.89±0.21^{de}	14.75	3.23±0.15^{ab}	198.16±3.82^{d}	3.61	1.05	0.21±0.00^{e}
	6.25	15.00±0.76^{bcd}	13.72±0.38^{bcd}	15.75	3.42±0.15^{a}	186.37±7.70^{d}	3.56	0.88	0.25±0.00^{abc}
	11.25	13.36±0.85^{de}	12.71±0.31^{de}	15.00	3.03±0.05^{bc}	184.90±7.19^{d}	3.58	0.72	0.23±0.00^{cde}

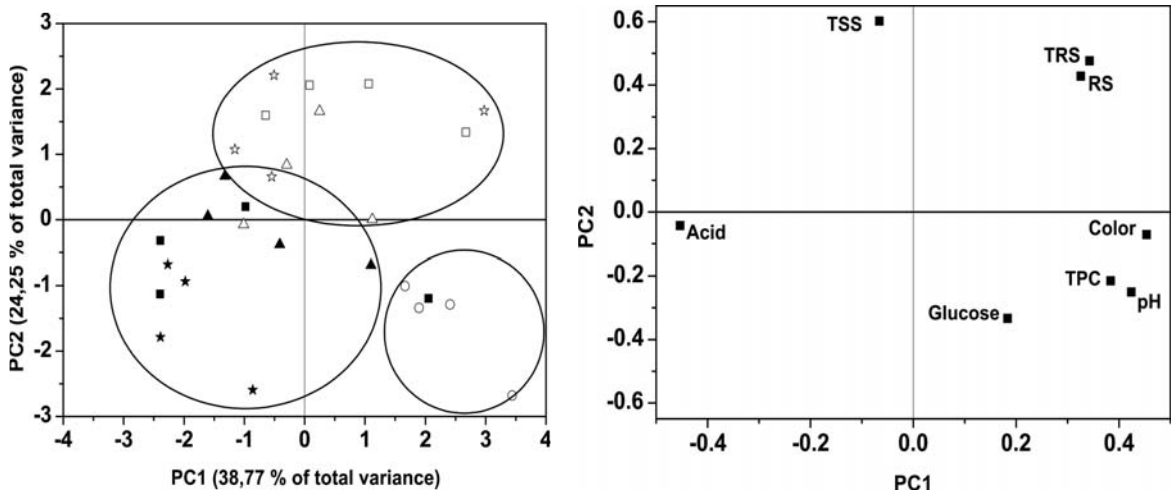
Note: T = temperature; C = concentration of enzyme; TRS = total reducing sugars; RS = reducing sugars; TSS = total soluble solids; TPC = total phenolic compounds. Data expressed as mean ± standard deviation. Values marked with the same letter are not significantly different ($p<0.05$). *Not variance analyses.

3.4 MULTIVARIATE ANALYSIS OF PHYSICOCHEMICAL DATA

Tables 2 and 3 show the values of the physicochemical analysis. In order to establish a clear connection between the samples and variables these data were submitted to Principal Component Analysis (PCA).

Figure 4(A) shows the score plot (PC1 vs. PC2) for the whole set of variables for an analysis with eight principal components. There was no variable exclusion, despite some of them having shown different behaviour for both enzymatic preparations. However, this kind of difference can be quite important if it can discriminate a product according to the preparation used in the process. Figure 4(B) shows the loadings plot, which highlights the variables according to their importance. Larger values of loadings were observed for TRS, RS, and pH.

The concomitant observation for scores and loadings values shows that the control samples were connected to higher contents of phenolic compounds, glucose, pH and colour. The samples processed with the Ultrazym®AFP-L demanded more acidity, while the juices produced with the Panzym®YieldMASH were related to higher contents of reducing sugar. These discriminations are related to the preparation method (conventional and with enzyme) and to the enzyme preparation (Ultrazym®AFP-L or Panzym®YieldMASH), but not to temperature.



(A) SCORES **(B) LOADINGS**

FIGURE 4 - PLOT OF SCORES (A) AND LOADINGS (B) FROM PCA ANALYSIS FOR JUICE OBTAINED BY CONVENTIONAL METHOD (○), AND WITH THE ENZYMES ULTRAZYM®AFP-L, 20 °C(■); 35 °C(▲); 50 °C(★); AND PANZYM®YELDMASH, 20 °C(□); 25 °C(△); 30 °C(☆)

TRS = total reducing sugars; RS = reducing sugars; TSS = total soluble solids; TPC = total phenolic compounds.

SIMCA modeling (Figure 5) was effective for both types of juice processing, traditional and enzymatic centrifugation, and the later are still split in two systems, Ultrazym®AFP-L and Panzym®YeldMASH enzyme. A set of samples was used to create the model and another set was used to validate it. There was good discrimination indicated by a clear formation of distinct classes for conventional processing and for each of the enzyme preparations. No superposition between classes was observed, in other words, samples that could present intermediate or different behaviour. The model obtained was tested with the set of samples used to validate it and no misclassification was observed, which confirms that the model was representative.

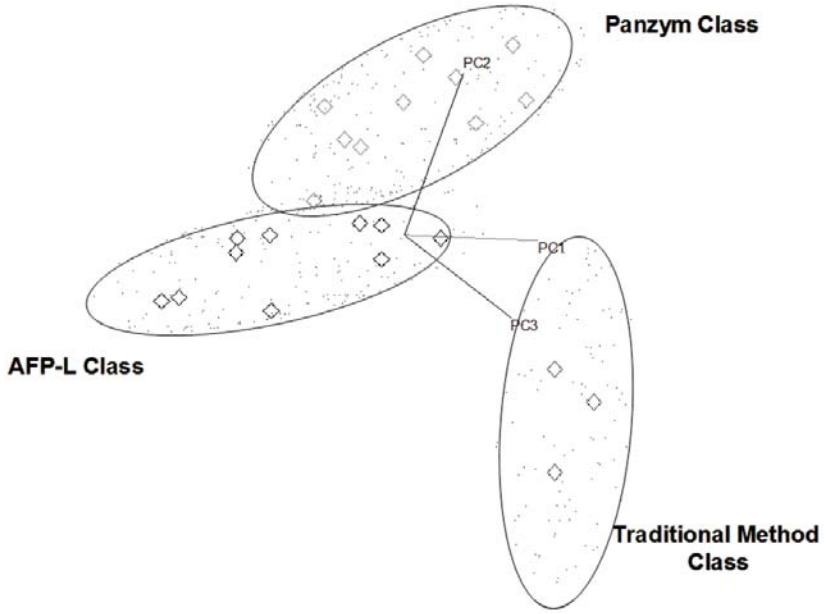


FIGURE 5 - SPATIAL DOMAINS OF BOTH ENZYME SETS AS COMPARED WITH NON-ENZYME CONTROL

Figure 6 shows the discrimination power for all the physicochemical variables in the development of the classification model. The less representative variable was the concentration of phenolic compounds. The remaining variables were quite significant and colour had the highest discrimination power. This lead to the observe that all the variables used were relevant in determining the differences between the type of process and enzymatic preparation.

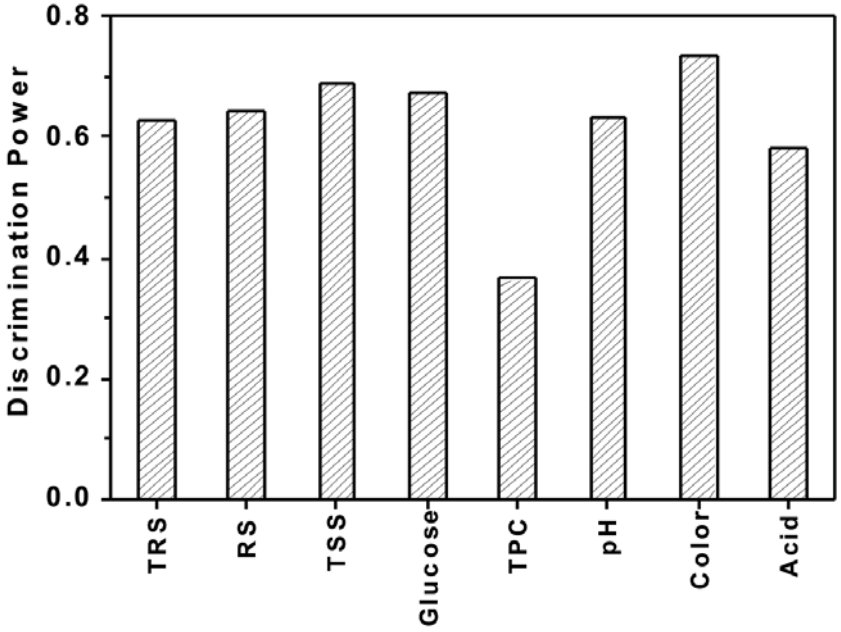


FIGURE 6 - DISCRIMINATION POWER OF VARIABLES FOR SIMCA MODEL FOR TRADITIONAL METHOD AND ULTRAZYM®AFP-L AND PANZYM®YELDMASH PREPARATIONS

4 CONCLUSION

Apple processing with industrial Ultrazym®AFP-L or Panzym®YeldMASH increases the juice yield and decreases the amount of pomace, depending on the enzyme concentration. The chemical composition was influenced both by processing and by the enzymes, which was confirmed by PCA. Traditional processing promoted higher contents of phenolic compounds and glucose. The Ultrazym®AFP-L enzyme favoured acidity, while Panzym®YeldMASH presented higher sugar content. SIMCA modelling showed the possibility of classifying the juices since it was possible to recognise patterns related to the process or to the type of enzyme.

RESUMO

TRATAMENTO DA POLPA DE MAÇÃ COM AS ENZIMAS ULTRAZYM®AFP-L E PANZYM®YIELDMASH

O objetivo deste trabalho foi verificar a influência das preparações Ultrazym®AFP-L e Panzym®YieldMASH no rendimento e na qualidade química de suco de maçã. Otimizou-se o processo de acordo com dois parâmetros, temperatura e concentração de enzima com tempo de reação de 1 hora. Os resultados das análises físico-químicas foram submetidos à análise estatística multivariada visando classificar os padrões. No modelo exploratório e classificatório, as amostras foram discriminadas com 100 % de correlação. Os resultados do planejamento experimental fatorial demonstraram maior influência da concentração enzimática para o aumento do rendimento. Os sucos tratados com as enzimas apresentaram rendimento cerca de 30 % maior que o controle. A observação dos dados mediante as ferramentas exploratórias da Análise de Componentes Principais (ACP) mostrou a tendência de separar as amostras controle pelos maiores teores de compostos fenólicos, glucose, pH e cor, as amostras tratadas com Ultrazym®AFP-L devido aos maiores níveis de acidez total e as amostras com Panzym®YieldMASH pelos altos teores de açúcares. Portanto, a aplicação de enzimas industriais no processamento de maçãs aumenta o rendimento de suco e diminui a quantidade de bagaço.

PALAVRAS-CHAVE: PREPARAÇÃO ENZIMÁTICA; MODELO EMPÍRICO; ANÁLISE MULTIVARIADA; ULTRAZYM®AFP-L; PANZYM®YIELDMASH; MAÇÃ.

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