

INFLUENCE OF THE SOLUBLE SOLIDS ON THE ZETA POTENTIAL OF A CLOUDY APPLE JUICE

E. I. BENITEZ and J. E. LOZANO

PLAPIQUI (UNS-CONICET), CC 717, (8000) Bahía Blanca, Argentina.

jlozano@plapiqui.edu.ar

Abstract-- The effect of soluble solids on the Zeta-potential (ξ) of a cloudy apple juice was investigated. Apple juice was enzymatically treated with pectinolytic enzymes and diafiltered with a polysulphone UF membrane (100 kDa) to eliminate soluble solids. Most soluble solids were removed after 4 equivalent sample volumes (ESV) of washing water. During a second period, from 5 to 23 ESV, diafiltration rate was significantly reduced. ξ (mV) moderately increased its absolute value with diafiltration during the first diafiltration period, changing at a rate of -1.1 mV/°Brix up to 0.3 °Brix. Results also indicated that after the only restitution of sugars ξ remained practically constant. When malic acid was restituted to a diafiltered juice, remarkable changes in Zeta potential occurred. As particles in this type of colloids are strongly negative, pH modification had a significant influence.

Keywords— Cloudy apple juice, diafiltration, soluble solids, Zeta-potential.

I. INTRODUCTION

Apple juice can be produced cloud stable with the application of suitable processes. The juice contains a large amount of fine particles and pectin, so that throughout the storage time only little cloud will be deposited. Turbidity shows a lesser intention to sediment due to the smaller particle size and dissolved pectins, which increase the viscosity of the juice. However, the composition of cloud particles has an influence on the stability of the juice.

Cloudy apple juice is a colloidal suspension where the continuous medium is a solution of pectin, sugars (fructose, glucose and sucrose) and malic acid. The dispersed matter is mainly formed by cellular tissue comminuted during fruit processing. Colloidal particles consist of proteins, lipids, neutral polysaccharides, pectin and other substances like minerals (Pecoroni and Gierschner, 1993; Dietrich *et al.*, 1996). The literature discusses different models for building a stable cloud particle. The cloud particles contain a core, consisting of protein, which is positively charged. These speculations are supported by those of Yamasaki *et al.* (1964) and Endo (1965); in which cloud particles containing a positively charged protein core, are surrounded by a

carbohydrate shell consisting among others of negatively charged pectin. This positively charged core is able to build a complex with negatively charged pectin. Moreover the formation of a protein-polyphenol complex was also discussed (Pecoroni and Gierschner, 1993). Cloud particles are modeled to consist of negatively charged partly demethoxylated pectin wrapped around a core of carbohydrates and positively charged protein (Beveridge, 1997). Depectinization may partially degrades pectin and expose protein beneath. Then aggregation between polycations and polyanions occurs, making possible the colloid flocculation. Both clarification of fruit juices with gelatin and cloudy juice stabilization with alginates confirm this model (Belitz and Grosch, 1997).

A protein's state of ionization is determined not only by the nature of its side chains but also by the pH of the solution environment. In acidic conditions proteins tend to acquire a net positive charge. In basic conditions, proteins tend to have a net negative charge. Between these extremes, at a precise value of the pH called the isoelectric point, the value of which is unique for each species of protein, the most thermodynamically stable form of the protein has equal numbers of positive and negative charges and does not migrate in an electric field (Magdassi and Kamyshny, 1996).

Colloidal particles accumulate charge at their surface that can be expressed as a surface potential. Surface potential is an important factor for determining the magnitude of charged-based colloidal interactions of a particle, most crucially electrostatic repulsion of other like charged particles. The surface charge on a particle perturbs the ionic distribution in the medium surrounding it. First a layer of tightly bound counterions (i.e. of opposing charge) accumulates at the particle surface, known as Stern layer, and beyond this a region of decaying excess concentration, the diffuse layer, extends a considerable distance into the surrounding aqueous media (McClements, 1999).

The repulsion is not a simple case of physical rebounding between particles. Repulsion occurs in part as a result of the interaction of the diffuse electrical double layer of charge produced between the particles. As the ionic strength of the juice increases, the thickness of the double layer decreases. When negatively charged particles approaches each other (both with double

electrical layers) mutually repulsive forces increase. Conversely, if the two surfaces can approach past the repulsive maximum, attractive London-van der Waals forces will take over, overcome the repulsive forces, and the particles attached each other (McClements, 1999). The high velocities imparted to colloids smaller than 0.1 microns due to Brownian motion provide the mechanism for overcoming the electrostatic repulsion of the double layers. The process is delicately balanced such that the reduction of the electrical double layer thickness through increased ionic strength is also required.

Surface potential can be indirectly measured through the Zeta potential (Ottewill, 1977). Zeta potential (ξ) is the electric potential, or charge, that exists between particles and the surrounding solution. Zeta potential is an important and useful indicator that this charge can be used to predict and control the stability of colloidal suspensions. The greater the zeta potential, the more likely the suspension will be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. Zeta potential is an electrical charge that describes how far apart colloidal particles are. Greater zeta potential indicates more space between particles. As a limit between stable and unstable suspensions, $\xi = \pm 30\text{mV}$ may be assumed (Sherman, 1970).

Aqueous colloidal systems, as a cloudy apple juice, are sensitive to the ionic strength and pH of the aqueous phase (McClements, 1999). According to the classic Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Overbeek, 1977), the stability of colloidal particles would depend on the balance between attractive van der Waals forces and repulsive electrostatic forces. While at low electrolyte concentration the energy barrier is strong enough to prevent particle aggregation, as the ion concentration is increased, the electrostatic interaction becomes effectively "screened" and aggregation and flocculation of particles may occur (Verwey and Overbeek, 1948). However, Genovese and Lozano (2005) found that colloidal characteristics of cloudy apple juice might be more realistically modeled with the extended DLVO theory, including repulsive hydration energies.

Soluble solids content indirectly alters the electrophoretic mobility of the apple juice, through changes in the viscosity of the serum. Reciprocal dependence of solute electrophoretic mobility (μ) on viscosity has been demonstrated (Li, 1992). Electrophoretic mobility was found not to be sensitive to small changes in the viscosity of juices in the range of "natural" soluble solids concentration (Genovese and Lozano, 2000).

The main objective of the present work was to determine the effect on Zeta-potential of the solutes present in a cloudy apple juice. Apple juice was enzymatically treated with pectinolytic enzymes to eliminate soluble pectin, and diafiltered to remove sugar, organic acids and minerals. Moreover, the effect of the restoration of major solutes was also considered.

II. MATERIALS AND METHODS

APPLE JUICE was kindly provided by the industry. Juice was enzymatically treated (Solvay 5XLHA; 20 mg/l, 2 h a 50° C) to eliminate soluble pectin, by following the hot technique (Toribio and Lozano, 1984). Aliquots of juices were pasteurized (90°C, 5 min). Soluble solids at 20°C were determined with a digital Abbe type refractometer, according to 932.12 AOAC (2000) method. Malic acid content was determined by titration in accordance with the method reported by the International Federation of Fruit Juice Producers (IFFJP, 1974). The concentration of Na, K, Ca and Mg was determined with a GBC AA Spectrophotometer (GBC Scientific Equipment, Victoria, Australia), after digestion with concentrated HCl.

A. Diafiltration

Soluble solids of cloudy apple juice were reduced by "washing" out with water, a process called diafiltration. This is essentially a dilution process and is performed in conjunction with a concentration process. Water is added while filtrate is removed. Apple juice was diafiltered (21.4L water per liter of juice) with a Ultrafiltration system (Osmonic Sepa[®] CF; Osmonics; Minnetonka; Mn; USA) using a 100 kDa (MWCO) polysulphone membrane. During diafiltration, juice aliquots were extracted at the different soluble solids content. Assays were performed in quadruplicate. Selected apple juice aliquots were also reconstituted by the adding of glucose, fructose, malic acid (Mallinckrodt Medical Argentina, Ltda.; Bs. As., Argentina) and the major cations as their salts (Anedra S.A., Bs. As., Argentina).

Both Zeta-potential (ξ) and electrical conductivity (ϵ) were determined with a Malvern Zetasizer 3000 (Malvern Instruments Inc., London). Obtained Zeta potential values are the result of 10 replicates.

III. RESULTS AND DISCUSSION

Characteristics of the assayed apple juice were summarized in Table 1. The major mineral ion and organic acid were potassium and malic, respectively. Soluble solids content was in accordance with previously reported values (Eisele and Drake, 2005). Aliquots of diafiltered juices were restituted in soluble solids content, to the amount reported in Table 1.

As Fig. 1 shows, during diafiltration of apple juice, quantified as the variation of soluble solids (°Brix) and electrical conductivity (ϵ) ($\text{mS}\cdot\text{cm}^{-1}$), two distinct exponential periods were identified. Most of soluble solids were removed during a first diafiltration period, up to 4 equivalent sample volumes (ESV) approximately. This relatively elevated rate of "washing" period could be represented using perfect mixing models (Loncin and Merson, 1979).

During the second period diafiltration rate was significantly reduced, behavior attributed to residual soluble solids more strongly "attached" as a laminar boundary layer both on juice particles and the surface of the ultrafiltration equipment. Under these conditions diafiltration was assumed to be diffusion controlled.

Table 1: Apple juice characteristics of interest for the study of Z-potential.

	Depectinized juice	Diafiltered juice
Soluble solids (°Brix)	12.5 ± 0.4	< 0.05
Conductivity (mS/cm)	2.84 ± 0.01	0.06 ± 0.01
Calcium (ppm)	64 ± 2	7.4 ± 0.2
Magnesium (ppm)	26 ± 2	1.5 ± 0.5
Potassium (ppm)	1058 ± 7	< 0.5
Sodium (ppm)	43 ± 5	1 ± 0.5
Malic acid (g/l)	6.96 ± 0.08	0.07 ± 0.01
pH	3.71 ± 0.02	4.55 ± 0.02

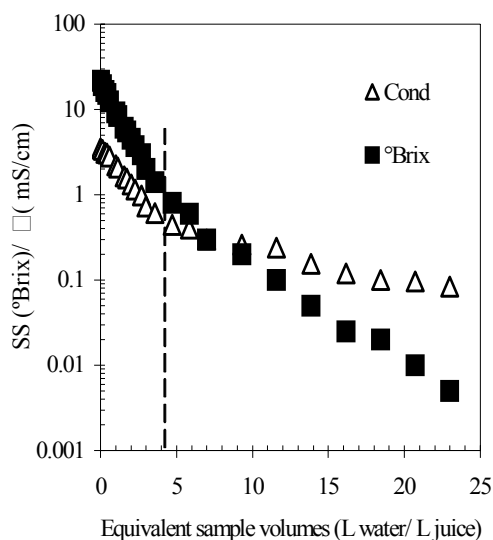


Figure 1: Variation of soluble solids (°Brix) and electrical conductivity (ϵ) ($\text{mS}\cdot\text{cm}^{-1}$) during diafiltration of apple juice.

Using continuous diafiltration most of permeable solutes were removed by washing through 6 equivalent sample volumes. However, the soluble solids that still remain in the juice, have a remarkable effect on Zeta potential. Therefore, diafiltration was continued until the electric conductivity (ϵ) remained constant at 0.06 mS/cm; approximately 23 EVS.

A. Effect of total soluble solids reduction by diafiltration

Figure 2 shows the effect of soluble solids, as °Brix, on the apple juice Zeta potential (ξ). It was observed that ξ (mV) moderately increased its absolute value in a linear way (becoming more negative), during the first diafiltration period, changing at a rate of 1.1 mV/°Brix. After that point (approximately 0.3 °Brix) ξ changed more suddenly, with a rate of increase of 25 mV/°Brix.

Change of ξ with the electrical conductivity (ϵ) (Fig. 3), more associated to the removing of natural salts present in apple juice, resulted exponential and reasonably well fitted to the following equation:

$$y = 5.2809 \ln(x) - 19.699 \quad (1)$$

$$r^2 = 0.985$$

On the other hand, decrease in malic acid content during diafiltration was individually determined, as the major organic acid present in apple juice, and results are shown in Fig. 4.

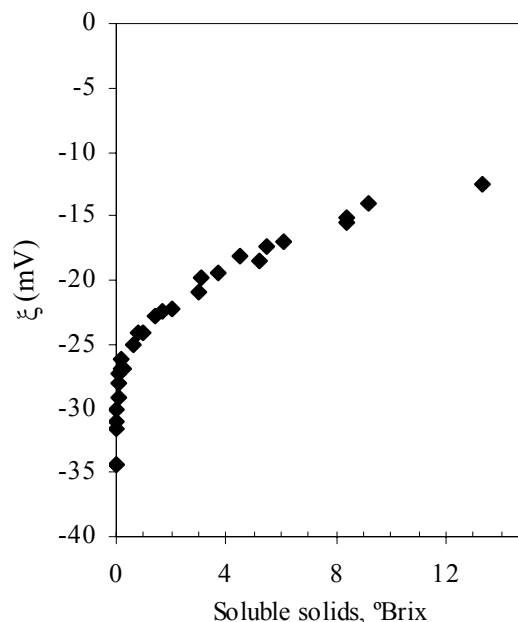


Figure 2: Effect of soluble solids (°Brix) on the apple juice Zeta potential (ξ).

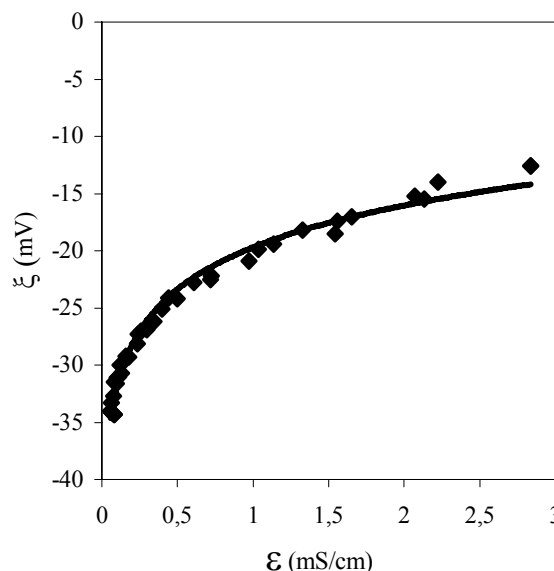


Figure 3: Change of ξ with the electrical conductivity (ϵ) during diafiltration of apple juice.

B. Effect of the restitution of soluble solids on the diafiltered apple juice Zeta potential

Figure 5 shows the variation in ξ with sugar addition (fructose and glucose, as the major carbohydrates in apple; Babsky *et al.*, 1986) to the diafiltered juice. Considering a simple Zeta potential expression (McClements, 1999) as a function of viscosity (η), electrophoretic mobility (μ); dielectric constant of vacuum (ϵ_0); and relative dielectric constant of the material (ϵ_r):

$$\xi = \eta \mu / \epsilon_0 \epsilon_r \quad (2)$$

and assuming that at the studied soluble solids concentration all parameters but viscosity remained practically constant, the effect of viscosity on Zeta

potential determination must be considered. When change in viscosity with soluble solids (Constenla *et al.*, 1989) was taken into consideration, results show that ξ remained practically constant after restitution of sugars to the initial values (Fig. 5).

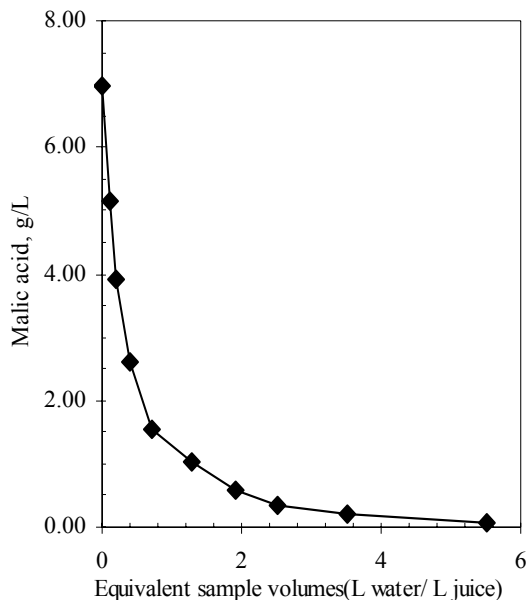


Figure 4: Decrease in malic acid content of an apple juice during diafiltration.

It is well known (Sennett and Olivier, 1965) that Zeta potential changes with salt concentration, potential determining ion concentration, and pH. The effect of pH on Zeta potential, due to the addition of malic acid, or strong acid and basis to the diafiltered juice was plotted in Fig. 6.

The isoelectric point (IP) of proteins has been located at pH= 3.0- 4.5 (Dietrich *et al.*, 1996). However, as Fig. 6 shows, apple juice particles remained negatively charged and no IP was determined, even at pH values as low as (pH=) 2. From this result it may be inferred that after pectinase treatment, particle surface is still negative, and no protein exposition was verified. Particles negative charge was attributed to degraded pectin molecules that remain bonded to the surface (Sorrivas *et al.*, 2006).

The magnitude of the zeta potential always "decreases" (in this case, becomes less negative) with an increase in electrolyte concentration. In particular, the zeta potential of cloudy juice becomes less negative as the NaCl, or KCl concentration in solution increases (Fig. 7), matching to conventional electric double layer theories.

This trend was expected since the increase of an indifferent electrolyte concentration decreases the thickness of electrical double layer, which is equal to the reciprocal of the parameter κ (nm^{-1}) (Debye-Hueckel parameter) (Hunter, 1986):

$$\kappa = 3.288 \sqrt{\frac{1}{2} \sum c_i z_i^2} \quad (3)$$

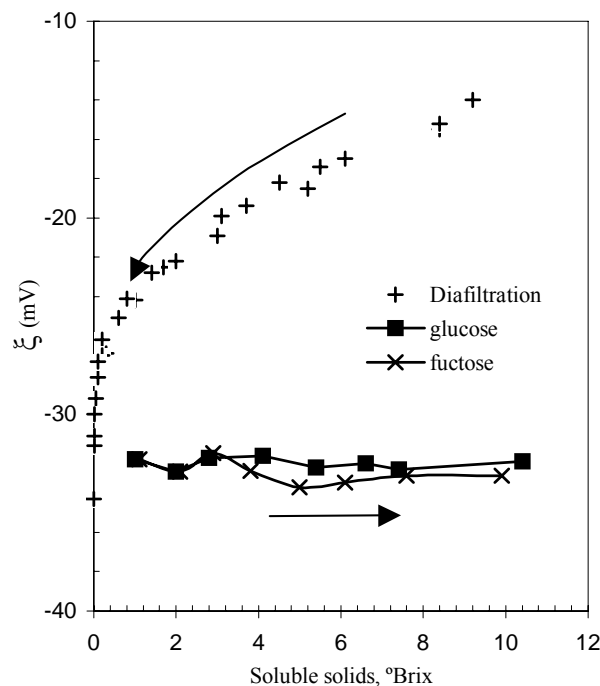


Figure 5: Increase in ξ with the addition of reducing sugar (fructose and glucose) to a diafiltered apple juice.

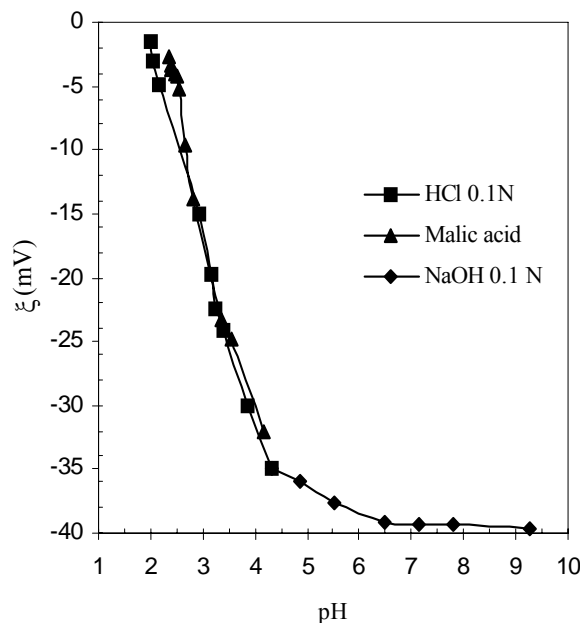


Figure 6: Effect of pH on Zeta potential (ξ), due to the adding of malic acid, or strong acid and basis to a diafiltered apple juice.

where, c_i and z_i are the concentration and valence of ion i , respectively. Increasing the ionic strength causes an increase in κ as a result of which the diffuse double layer shrinks closer to the particle and the potential falls off more rapidly with distance. This is referred to as 'compression of the double layer'.

However, the initial ξ values of the cloudy apple juice were not recovered by the single addition of mineral ions to the diafiltered juice. It is clear that the influence of mineral ions on the stability of colloidal systems as cloudy apple juice is complex and depends

on the precise nature of the ions, the electrical characteristics of the particles surface and the prevailing environmental conditions (McClements, 1999). It was speculated that Na^+ , K^+ and Cl^- ions are indifferent ions in the pectin-protein particle stabilized suspension. Therefore the reduction of ξ with the increase of the ionic strength was only attributed to the contraction of the electrical double layer around the charged particles.

Figure 8 shows the effect of malic acid on ξ , when added to a diafiltered cloudy apple juice. As particles in this type of colloids are strongly negative, pH modification implies strong changes in the Zeta potential (Fig.6). Finally, Fig. 9 shows the effect of the adding of malic acid, with different concentration of KCl. It was observed that for a concentration of 0.009M KCl, diafiltration and recovering curve are practically coincident.

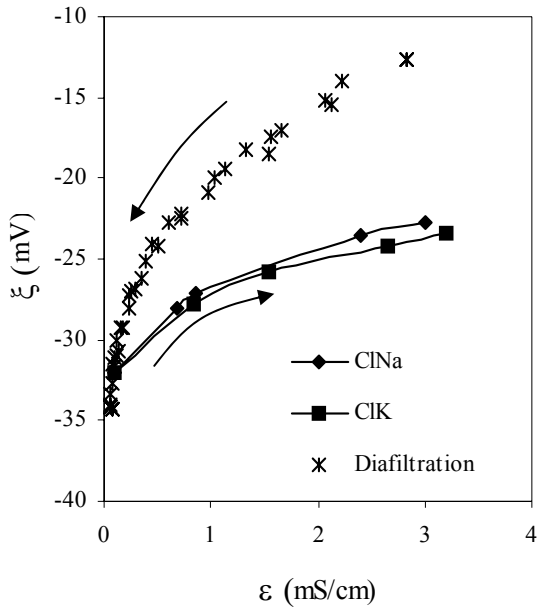


Figure 7: Variation of the Zeta potential (ξ) of a apple juice with salt concentration (NaCl and KCl).

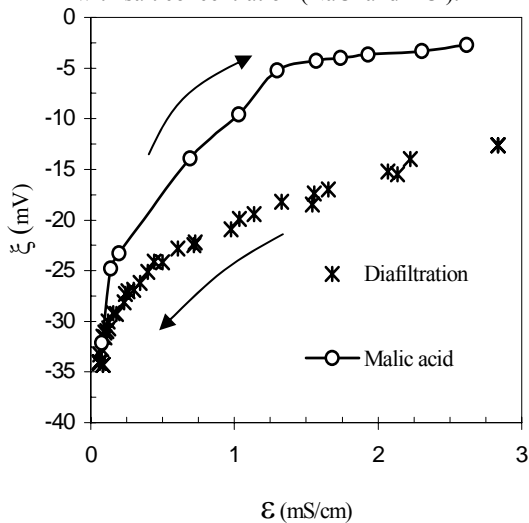


Figure 8: Effect of malic acid on ξ , when added to a diafiltered apple juice.

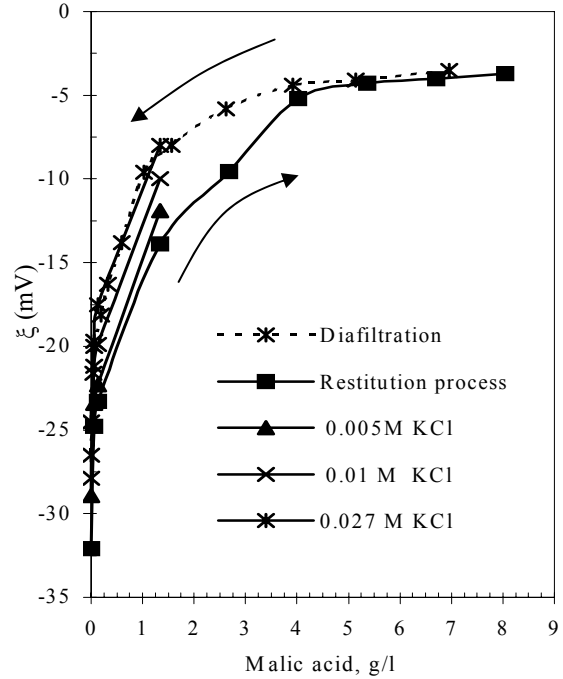


Figure 9: Effect of the adding of malic acid, with different concentration of KCl, on the Zeta potential of a diafiltered apple juice

IV. CONCLUSIONS

This study shows that cloudy apple juice particles are negatively charged, depending on the concentration of the dissolved species. After the only restitution of sugars Zeta potential (ξ) remained practically constant. As apple juice particles are strongly negative, pH modification had a significant influence. When malic acid was restituted remarkable changes in Zeta potential occurred. Based on these results, the following observations were made: (i) the zeta potential is negative at all pH values investigated; (ii) the zeta potential becomes more negative as the pH increases; and (iii) cloudy apple juice remained as a stable colloid, despite the enzymatic treatment. This last behavior was attributed to the ineffectiveness of pectinases to degrade pectin attached to particles. It is conjectured that, in addition classical DLVO inter-particle forces, other repulsive energies as the hydrophilic interaction must be considered.

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