EFFECT OF GELATIN ON APPLE JUICE TURBIDITY

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***Abstract*— Clarification of apple juice by flocculation and precipitation with bentonite and gelatin is explained on a more systematic basis, essentially through the determination of turbidity and zeta-potential. Apple juice was also treated with Polyvinylpolypyrrolidone (PVPP) to remove total polyphenol. Gelatin-particle complex was evaluated as the increase in juice turbidity after the adding of tannic acid. The change of slope during Zeta potential determination indicated that electrostatic forces predominate at low gelatin content, and hydrophobic and hydrophilic interactions occur later at a higher gelatin content. Results also indicated that tannic acid test is useful for determining optimal gelatin concentration for clarification, as test was unaffected by soluble solids, acidity, or pectinase treatment. Gelatin consumption was mainly attributed to colloidal particles. Finally, results indicated that risk of haze by free gelatin in juice required at least 10 times more gelatin than the optimum dosage for clarification.**

***Keywords*— bentonite, gelatin, polyphenol, PVPP, tannic acid, apple juice clarification.**

I. INTRODUCTION

Cloudy apple juice is a colloidal suspension where the continuous medium is a solution of pectin, sugars and malic acid, and the dispersed matter is mainly formed by cellular tissue comminuted during fruit processing. A colloid is a suspension in which the dispersed phase is so small (~1-1000nm) that gravitational forces are negligible and interactions are dominated by short-range forces, such as van der Waals attraction and surface charges. The inertia of the dispersed phase is small enough to exhibits random Brownian motion, driven by momentum imparted by collisions with molecules of the suspending medium.

For obtaining a clear juice these suspended particles have to be removed. This process is known as clarification, or fining, one of the most important unit operations in apple juice processing. To obtain a completely transparent liquid, suspension must be firstly un-stabilized. This procedure helps also to remove active haze precursors, decreasing the potential for haze formation during storage and providing a more limpid juice (Hsu *et al.*, 1987; 1989; 1990). Therefore, the fining step is an important procedure that should be carefully controlled during the processing of clarified apple juice.

Conventional enzyme clarification appears to be a critical processing step which, if excluded, may result in the formation of larger quantities of haze (Tajchakavit, *et al.*, 2001). Enzymatic treatment also allows an efficient use of clarifying agents to assist with cloud removal. Addition of fining, or clarifying, agents is intended to modify clarity, color, flavor and/or stability of juices. They are grouped according to their general nature in (i) Earths (bentonite, kaolin); (ii) Proteins (gelatin, isinglass, casein, albumen); (iii) Polysaccharides (agars); (iv) Carbons; (v) Synthetic polymers (PVPP, nylon); (vi) Silicon dioxide (kieselsols); and (vii) Others, including metal chelators, enzymes, etc. (Zoecklein, 1988).

Clarification of apple juice with gelatin and bentonite is a common industrial practice (Stocké, 1998). These fining agents work either by sticking to the particles, or by using charged ions to cause particles to stick to each other, in any case making them heavy enough to sink to the bottom by the action of gravity. What is left is a transparent though not a clear juice. Subsequent filtration operations are needed to obtain a crystal clear product. Differences in the nature of ionic charges of protein, polyphenols and the fining agents, induce flocculation and sedimentation and result in the removal of these potential haze precursors from solution.

Both tannins and anthocyanins in fruit juices are proposed as the major source of the hydrogen bonds, which are the basis of complex formation between gelatin and tannins or anthocyanins. Haze-active polyphenol may increase consumption of gelatin during fining.

Determination of appropriate doses of clarifying apple juice agents (bentonite, gelatin) is usually made at the industry by trial and error: basically, in a matrix of test tubes filled with enzymatically treated juice, increasing quantities of bentonite and gelatin are added in rows and columns, respectively. The dose that in a shorter time gives the most compact flocks and transparent supernatant is selected for the bulk treatment of juice.

During the last years conventional clarification process is being replaced by the use of ultrafiltration membranes (Alvarez *et al.*, 1998). However clarification of fruit juice by ultrafiltration alone does not remove active haze precursors, allowing haze formation during storage. Bentonite, Polyvinylpolypyrrolidone (PVPP) and activated charcoal are used to eliminate natural polyphenols present in fruit juices (Kwang-Sup *et al.*, 2004). It was also claimed that gelatin, contrarily to PVPP which eliminate all fruit juice polyphenols, would only eliminate post-bottling haze forming polyphenols (Siebert and Lynn, 1997). For that reason gelatin is a frequently used clarifying agent by fruit processing industries at the present.

A variety of factors must be considered in the selection of the type of gelatin and bentonite used for clarification. Moreover, excessive fining can be detrimental as this may result in the introduction in the juice of potential haze precursors, such as proteins. On the other hand, underfining could also contribute to haze development, as high level of polyphenols and proteins may remain in the juice. The objective of the present work was to obtain more information on (i) the gelatin interaction with apple juice components, and (ii) the mechanism of gelatin clarification.

II. MATERIAL AND METHODS

Cloudy apple juice was kindly provided by the industry (Jugo S.A., Villa Regina, Rio Negro, Arg.). Juice was enzymatically treated (Solvay 5XLHA; 20 mg/l, 2 h a 50ºC) to eliminate soluble pectin, by following the hot technique (Toribio and Lozano, 1984). The following juice samples were prepared:

CAJ: Apple juice clarified by ultrafiltration (permeate).

CAJ+ PVPP: Clarified apple juice treated with Polyvinylpolypyrrolidone (PVPP) and bentonite to remove natural occurring polyphenols in apple juice.

DJ: Diafiltred juice; practically apple juice particles in water.

MCJ: Modeled Cloudy Juice; juice clarified by ultrafiltration, with a known fraction of particles.

DJ+P: Diafiltred juice plus hydrolyzed pectin.

DJ+MA: Diafiltred juice plus malic acid.

DJ+PMA: Diafiltred juice plus malic acid and hydrolyzed pectin.

DJ+G: Diafiltred juice plus glucose.

Apple juice was diafiltred (21.4L water per litter of juice) with an Ultrafiltration system (Osmonic Sepa® CF; Osmonics; Minnetonka; Mn; USA) using a 100 kDa (MWCO) polysulphone membrane. Diafiltration was continued until the electric conductivity (ε) remained constant at 0.06 mS/cm (Benítez and Lozano, 2006).

Juices with known initial mass fraction of particles (Co) were determined by freeze-drying of diafiltred juice. Diafiltred juice (25 mL) was freeze dried in a HETO Model FD 8.0 (Heto-Holten, Denmark) freeze dryer. The heating plate temperature was set to 20°C and the vacuum to 0.1 mBar to initiate drying. After drying for 48 hrs, apple juice particles were removed from trays and stored under vacuum in desiccators with P_2O_5 until use. The necessary amount of sample juices with Co=0.303 mg particles per liter, was prepared. Fig. 1 schematically represents the methodology followed in the present study. Description of assays indicated in Fig. 1 follows:

(1) *Zeta potential* (ξ) *and the effect of gelatin*. The objective of this assay was to determine the effect of individual apple juice components (isolated particles; malic acid; glucose as representative carbohydrate; and rests of pectin after enzymatic treatment) on ξ, and how it changes after the adding of gelatin. At juice pH, while particles are negatively charged, gelatin charge is positive. As a result, a reduction on net surface charge is estimated. That change may be used to determine minimum gelatin concentration during the clarification process.

(2) *Conventional clarification assay*. Conventional clarification test, performed with an arrange of apple juice test tubes with increasing bentonite/gelatin content addition, was made to confirm that gelatin dose determined in the previous step actually conduce to juice clarification.

(3) *Gelatin test (Polyphenols effect*). It is known that gelatin binds not only to colloidal apple particles but to polyphenols, naturally present in the juice, increasing turbidity. Effect of polyphenols in gelatin consumption was evaluated considering turbidity changes in clarified (CAJ) and modeled (MCJ) apple juice.

(4) *Tannic acid Test (Effect of Polyphenols).* The tannic acid test (TAT) is useful to evaluate non-bonded gelatin after the conventional clarification assay. Free gelatin will produce haze with tannic acid. More over, increase in turbidity by TAT in a CAJ sample shows the effect of polyphenols. If haze appears in an ultrafiltred juice previously treated with PVPP and bentonite, the only responsible is gelatin (proteins and polyphenols were removed). On the other hand TAT applied to a diafiltred juice (DJ) may reflect the effect of the isolated particles. Finally, in a modeled juice the test of tannic acid is a tool to evaluate the effect of both proteinaceous particles and turbidity in haze formation.

Soluble solids were measured in degrees Brix with a bench refractometer AO Scientific, Mark II, at 20 (+0.l)°C. All chemicals were analytical grade from Sigma Chem. Co. (St. Louis, MO). Turbidity was determined with a PC Compact Turbidimeter (Aqualitic, Germany) as Nephelometric Turbidity Unit (NTU). Samples of the juice were placed in a 15 ml cell, capped and gently inverted twice to ensure even mixing. Zeta-potential (ξ) and electric conductivity (ε) were determined with a Malvern Zetasizer 3000 (Malvern Instruments Inc., London). Obtained Zeta potential values are the result of 10 replicates. Malic acid content was determined by titration in accordance with the method reported by the IFFJP (1974). Initial malic acid content resulted 6.33± 0.01 g/L.

Gelatin (Cristagel Stauffer, Type A, 175 Bloom) was used as clarifying agent. For fining a 2% solution of 0.64±0.02 g protein/g gelatin, as determined with the Biuret reaction (Gornall *et al.*, 1949) was prepared. A 5% weight/volume commercial sodium bentonite type I (La Elcha; Mendoza, Argentina) suspension were prepared in hot water (60°C). Tannic acid (TA) was purchased from Sigma- Aldrich (Germany). Tannic acid stock solutions (10 g/L) was prepared by dissolving TA in 20 mL ethanol (95%), and diluting to 100 ml with distilled water.

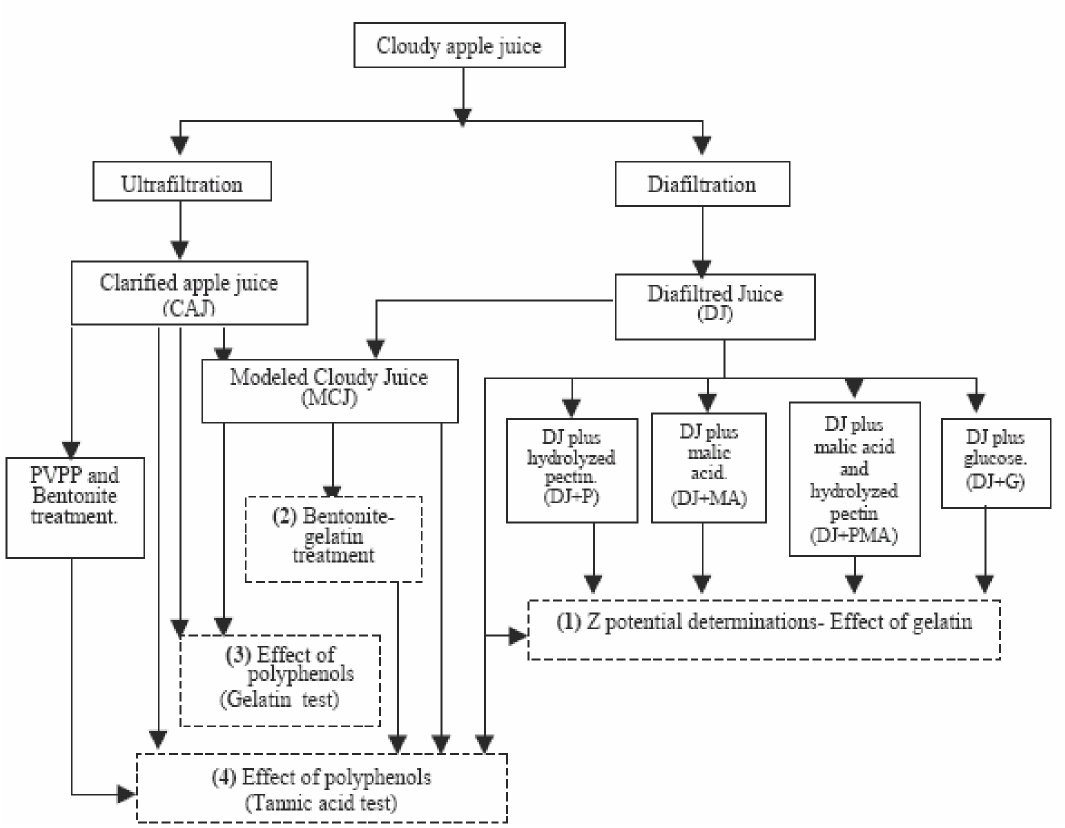

Figure 1: Flow diagram of clarification studies procedure.

Gelatin dosage was made to 50 mL apple juice test tubes with increasing gelatin content addition (0.002; 0.006; 0.017; 0.03 and 0.05). After the addition of gelatin, test tubes were agitated with a magnetic stirrer for 30 seconds. Zeta-potential was determined after 10 min.

The effect of soluble solids on gelatin clarification was studied by adding glucose solution (100g/L) to a concentrated diafiltred juice (DJ) to obtain a final mixture (glucose- DJ) with Co= 0.303 mg/L particles and 10ºBrix. The effect of organic acids on clarification was studied by the adding of malic acid at the original (DJ) apple juice concentration.

A. Pectin adding and enzymatic treatment

In order to evaluate the effect of degraded pectin on gelatin clarification, hydrolyzed pectin solution was added to a concentrated diafiltred juice (DJ) obtaining a final mixture (100 g/L) with Co= 0.303 mg/L particles.

B. Conventional gelatin/bentonite clarification assays

Conventional method for bentonite and gelatin dosage was made with an arrange of 4 columns of 50 mL apple juice test tubes with increasing gelatin content addition (0; 0.016; 0.032; 0.048 and 0.06) and 4 rows of tubes with increasing addition of bentonite (0.16; 0.32; 0.48; 0.64), and assuming ten fold the necessary amount of bentonite required to eliminate the added gelatin (Van Buren, 1989). Firstly gelatin was added and tubes let to rest for 1 min. Then, the corresponding dose of bentonite was added and let to settle for 30 min. After the addition of each clarifying agent, tubes were gently agitated for a few seconds. Once flocculation and sedimentation were completed, supernatant was carefully siphoned from every tube, filtered though Whatman #3 paper and turbidity determined.

C. Determination of Haze-Active Protein

It was based in the Siebert and Lynn (1997) procedure. Polyphenols and native proteins are removed with PVPP and bentonite respectively. Then tannic acid was added to increasing gelatin quantities and turbidity (NTU) was determined. In a juice free from polyphenols and native proteins, increase in turbidity with TA is only attributable to free gelatin in solution.

D. Data collection and analysis

Clarification assays were done at least in triplicate. All results reported are the average of replicate measurements. Values shown on graphs are means of replicate measurements.

III. RESULTS AND DISCUSSION

Figure 2 shows the effect of gelatin on Zeta-potential (ξ), when added to the different apple juice, and modeled juice solution, assayed. Results indicated that at gelatin doses lower than 0.05%, ξ drastically changed its value from the most negative to potentials about -10 mV, and even crossing to the positive side of the plot, in the case of diafiltred juices with malic acid (DJ+MA and DJ+PMA). For higher doses of gelatin ξ practically reach a plateau, behavior attributable to an incompletely neutralization of the particle surface charges.

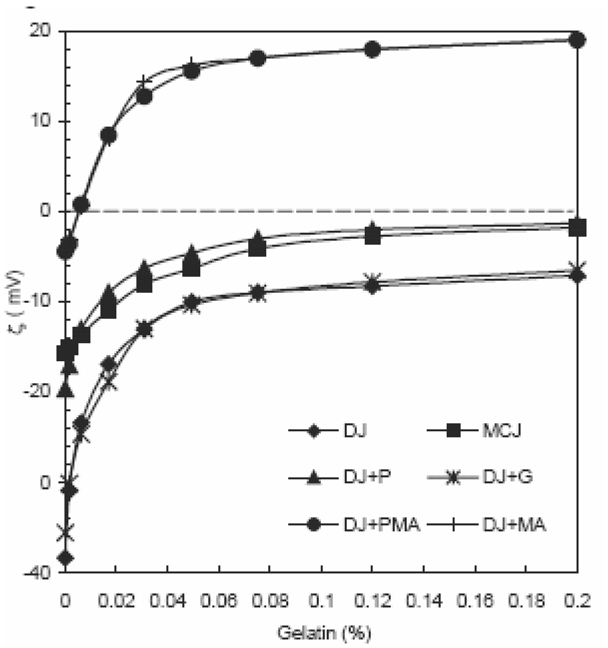

Figure 2: Effect of gelatin on the Zeta potential (ξ) on the different apple juice and modeled juice solution assayed.

This result seems to indicate that gelatin consumption is mainly a function of the colloidal particle content, rather than the chemical composition of solution (soluble solids, acidity, degraded pectin, etc.). Moreover, as malic acid is an effective charge neutralizer (Benítez and Lozano, 2006), its presence implies a reduction in ξ values. Furthermore, the adding of positively charged gelatin facilitates the formation of gelatin-particle complex (Tostoguzov, 2003), attributable to the coexistence of gelatin and residual pectin molecules on particles surface. As a result ξ became exceptionally positive in this case (malic acid added). However, ξ remained negative for modeled cloudy juice (permeate+ particles), which include the malic acid content naturally present in apple juice. Discrepancy may be attributable to apple polyphenols remaining in the juice after ultrafiltration but removed in diafiltred samples.

Figure 3 shows how the bentonite-gelatin test affects turbidity of a MCJ sample (juice clarified by ultrafiltration, with a known fraction of particles). Results indicated that at concentrations of bentonite >0.32 juices was practically clarified (NTU≈ 0) at any of the assayed gelatin concentrations greater than 0.02%. Moreover, at lower bentonite concentration (<0.32%) the amount of gelatin necessary to drastically reduce turbidity was found to be 0.032%.

However in this last case, an excess of proteins remained in solution, as tannic acid test showed (Fig. 4).

Whether proteins where those naturally present in the apple juice, or resulted from non-precipitated gelatin, was not verified. For constant bentonite content an increase in turbidity should be expected due to the presence of non-combined gelatin. However, as turbidity was practically reduced to zero, apple proteins, and not the gelatin appeared to be responsible for the positive tests.

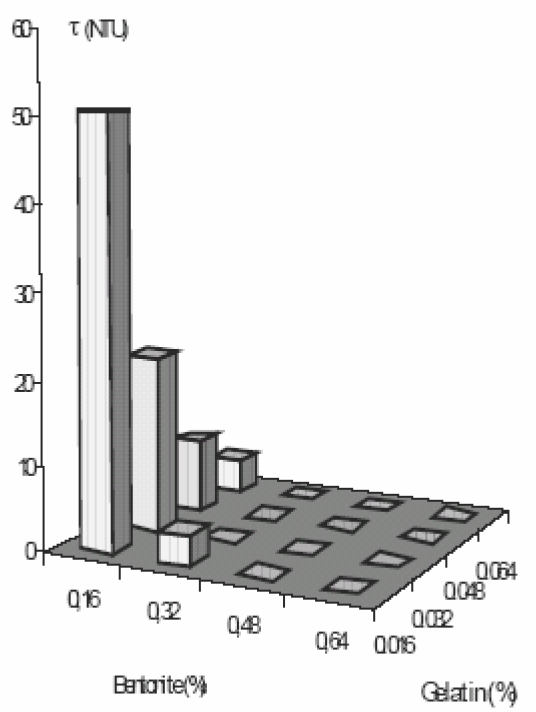

Figure 3: Effect of bentonite and gelatin on the turbidity of a modeled cloudy juice (juice clarified by ultrafiltration, with a known fraction of particles).

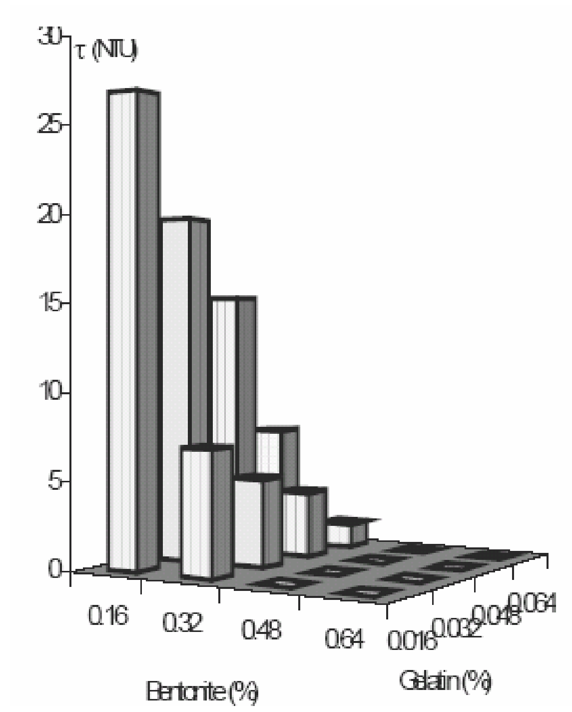

Figure 4: Effect of tannic acid on the turbidity of a modeled cloudy juice previously treated with bentonite and gelatin.

The amount of haze-active gelatin in apple juice was determined by adding tannic acid to induce haze followed by turbidimetry.

Figure 5 shows the effect of tannic acid on the turbidity of modeled (MCJ) juice after particle removal by bentonite-gelatin clarification, for increasing gelatin content keeping bentonite constant (0.30%). It was observed that tannic acid test resulted positive when gelatin content largely exceed the minimum quantity to produce a complete apple cloud flocculation.

The moderate increase in turbidity for gelatin content < 0.3% was attributed to non-flocculated particles still remaining in suspension due to inappropriate bentonite treatment. Results presented in Fig. 5 indicated that TAT was useful to evaluate the effect of colloidal particles on the formation of gelatin complexes.

Figure 6 shows the increase on turbidity with gelatin in permeated (CAJ); diafiltred (DJ) and modeled cloudy (MCJ) apple juice. The ratio of gelatin to polyphenol influences the amount of haze formed; that was explained by Siebert *et al.* (1996) using conceptual protein-polyphenols binding model. It was observed that in the case of CAJ turbidity increased with gelatin until a maximum (0.015% gelatin), identified with the point where active site of polyphenols equals active site of gelatin.

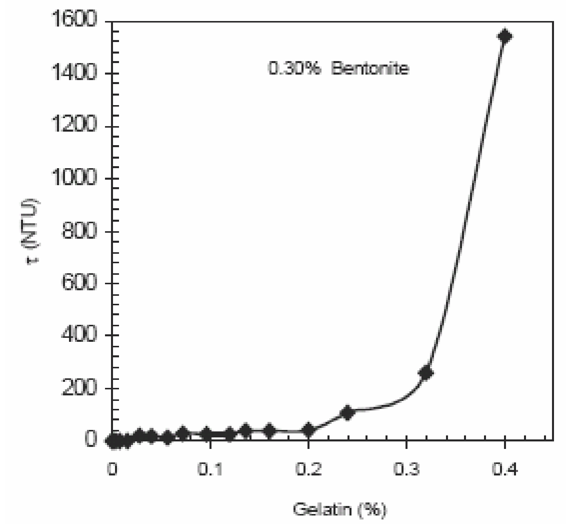

Figure 5: Effect of the excess of gelatin on the turbidity of diafiltred juice (DJ), as verified with tannic acids.

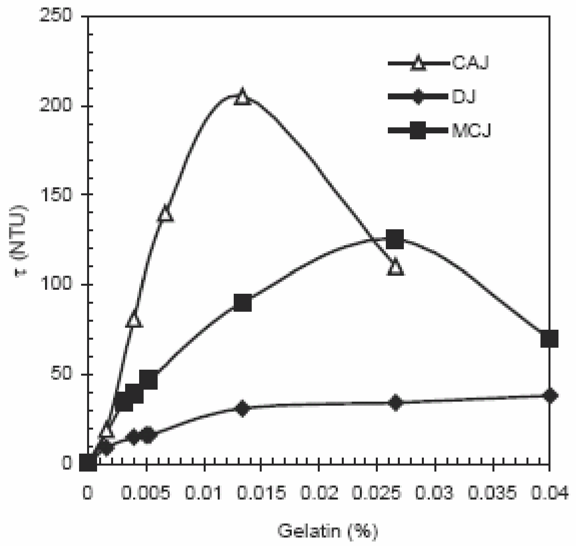

Figure 6: Changes in turbidity produced by the adding of gelatin in permeate (CAJ); diafiltred (DJ) and modeled (MCJ) apple juice.

The same maximum in turbidity was observed when gelatin test was assayed in MCJ samples. Contrarily to CAJ, in MCJ samples colloidal particles are present, and maximum turbidity was reached at a higher gelatin contents (0.027%). As flock precipitation was not observed in the previously described assays, reduction in turbidity was attributed to the reduction of natural occurring polyphenols in apple juice.

Figure 7 shows the effect of tannic acid on the turbidity of diafiltred (DJ); modeled cloudy (MCJ); permeated (CAJ) and permeated treated with PVPP + bentonite apple juice, previous gelatin treatment. PVPP treatment of apple juice effectively removed endogenous polyphenols. Results indicated that turbidity continuously increased with gelatin. However, while modeled (MCJ) and diafiltred (DJ) juice turbidity approach to a maximum, in permeate (PJ) juices the turbidity was nearly linear with gelatin concentration. On the other hand, CAJ treated with PVPP verified a lower turbidity than the untreated sample, attributable to the removal of haze-active polyphenols resulting in more “free” (non-bonded) gelatin in solution. Moreover, particles in DJ and MCJ samples also contributed to the reduction of active gelatin sites, resulting in a smaller turbidity. Being apple juice particles highly hydrophilic (Benítez *et al.*, 2006), suspension remained stable even for gelatin concentration > 0.03%.

IV. CONCLUSIONS

Apple colloidal particles bonded to gelatin reduced their surface charge at the pH of juice. Gelatin-particle complex can be evaluated as the increase in juice turbidity after the adding of tannic acid. Besides electrostatic interaction among particles and gelatin, hydrophobic and hydrophilic forces contribute to the stability of particle-gelatin complexes.

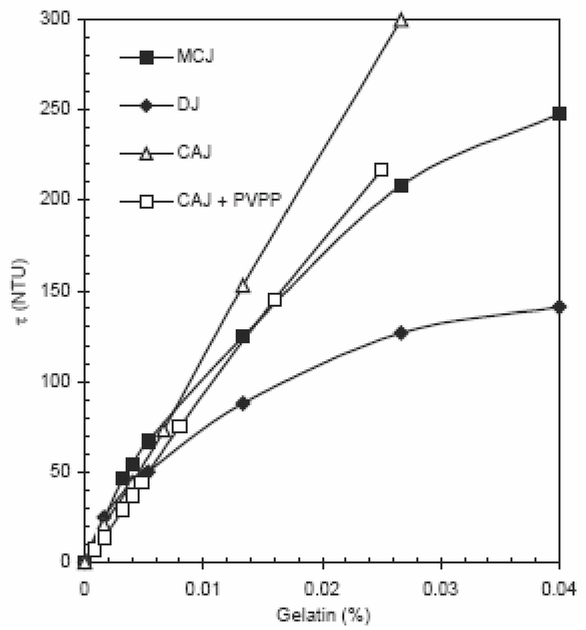

Figure 7: Changes in turbidity produced by the adding of tannic acid in gelatin treated diafiltred (DJ); modeled (MCJ); permeate (CAJ); and permeate treated with Polyvinylpolypyrrolidone (CAJ+ PVPP) apple juice.

The change of slope during Zeta potential determination seems to indicate that electrostatic forces predominate at low gelatin content and hydrophobic and hydrophilic interactions occur later at higher gelatin content. Obtained results may explain while it was possible to detect free gelatin in juice only at very high concentration. Finally, results also indicated that tannic acid test (TAT) was useful for determining optimal gelatin concentration for apple juice clarification. Moreover, TAT resulted unaffected by soluble solids, acidity, and enzymatic hydrolysis of pectin.

The effect of natural occurring polyphenols in gelatin clarification depends on polyphenols-to-particle ratio. In the case under study, gelatin consumption was mainly attributed to interaction with colloidal particles. Finally, results indicated that risk of haze by free gelatin in juice required at least 10 times more gelatin than the optimum adding for clarification.

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