THE AFFECT OF CALCIUM CHLORIDE CONCENTRATION AND pH ON THE CLOTTING TIME DURING THE RENNETING OF MILK

SÜTÜN RENNET İLE PIHTILAŞTIRILMASINDA KALSİYUM KLORÜR DERİŞİMİ VE pH'NIN PIHTILAŞMA SÜRESİNE ETKİSİ

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ÖZET: Kalsiyumun, rennet ile pıhtılaşma süreci üzerindeki etki mekanizması hala tam olarak açıklanamamıştır. Kimi araştırıcılar kalsiyumun etkisinin, eklenen kalsiyum klorür ile yalnızca pH'da düşüşe yol açmasıyla sınırlı olduğunu savunmaktadırlar.

Bu çalışmada, anılan etkisinin ötesinde, eklenen kalsiyumun yalnız agresyon tepkimesini değil aynı zamanda rennetlenme sırasındaki proteolizin hızını da etkilediği, kalsiyumun pıhtılaşma süresi üzerindeki etkisinin pH'yı düşürmesinden bağımsız olduğu saptanmıştır.

ABSTRACT: The mechanism behind the effect of calsium on renneting process has not been fully elucidated. Some of the authors are suggested that the affect of calcium on renneting was limited with decreasing of pH with additional calcium content. We also obtain that additional calcium content is affected not only the aggregation reaction but also the rate of proteolysis in renneting process of milk and the affect of calcium on clotting time was independent from dropping in pH. In this study, it is found that pH was an important factor for both of renetting reactions. During the rennetlng there is an interrelationship between pH and rennet clotting time. It is observed that there was a logarithmic relation between them.

INTRODUCTION

It is well known that the renneting of the milk consist two stages which were splitting of kappacasein by a proteolytic enzyme and flocculation of parakappa-casein micelles.

It is suggested that there was a ratio between the rate of enzymatic reaction and hydrogen ion activity, near 6.6 pH, and it was not effected by calcium, ions, but needs ionic strength (WALSTRA and VLIET, 1986). During the renneting process, para-casein micelles loose the colloidal protective aspect of kappa-casein by hydrolysis and they flocculate. The rate of flocculation reaches with calcium ion activity, increasing of the colloidal calcium phosphate (CCP) and there is not a significant dependence to pH (at the constant calcium ion activity). But for low pH degrees, this phenomenon deviates from above mentioned assumption (WALSTRA and VLIET, 1986).

The mechanism behind effect of calcium on renneting process has not been full explained. It is suggested that the aggregation was accelerated by the adsorbed cations which shielded the negatively charged groups of casein (HOOYDONK et al., 1986a). Adsorption of cations might be increased the hydrophobicity of rennet-converted micelles and promote aggregation.

DALGLEISH (1983) is also assumed that acceleration effect of calcium was due to the specific interactions of unknown nature and it was not a simple charged reduction of converted micelles.

The aggregation of para-casein micelles are integrated by completed hydrolysis of kappacasein, during the clotting. Rate of hydrolysis is reached with lower calcium chloride concentration but the concentration above 8 mM, increasing of calcium chloride has caused a considerable decrease of rate [4].

Clotting time is decreased by addition of calcium chloride at the initial stages and there is a minimum at 0.4M $CaCl_2$ concentration. Below this concentration, clotting is retarded. The rate of firming is also increased with $CaCl_2$. If the concentration would be higher than this, gel firmness could be decreased (McMAHON et al., 1984).

For obtain a normal renneted-gel, both of calcium ion activity and concentration of CCP in micelle have to be above on a minimum level (McMAHON et al., 1984). Importance of micellar calcium phosphate (MCP) either than calcium ion concentration, was determined by HOOYDONK et al. (1986a), hence removal MCP accelerated the renneting process.

It is suggested that the release of NPN during the renneting of calcium depleted milk was independent from calcium ion concentration (HOOYDONK et al., 1986a) However GREEN and MARSHALL (1977) observed that the rate of proteolysis was increased after addition of calcium or divalent cations.

It is well known that addition of calcium chloride to the cheese milk accelerates the renneting process. The acceleration is due to combined effect of the increased calcium concentration and dropped in pH (WALSTRA and VLIET, 1986). The shorter gelation times of the non adjustable sample in pH is partly due to the increased rate of enzymatic reaction with decreasing pH (HOOYDONK et al., 1986b). It is suggested that addition of calcium was not effected the rate of enzymatic reaction, when the pH kept constant (WALSTRA and VLIET, 1986). When calcium chloride content was reached from 3 mM to 60 mM, paracasein micelles were formed at lower hydrolysis degrees of kappa-casein, it was dropped from 71% to 27% (BRINGE and KINSELLA, 1986).

The shortening of the gelation time and renneting time are entirely due to an increase in the rate of aggregation. On the other hand drastic changes in the casein micellar system are brought about by acidification of milk, especially in the pH range 5.0 to 6.0. Small changes of the pH in 6.6 region have a significant effect on both of enzymatic and coagulation reactions. Around pH 6.3 the effect of small changes in acidity on the enzymatic reaction is less pronounced but the rate of gelation still increases gradually with decreasing pH. The maximum in curd-firming rate was found around pH 5.8 (HOOYDONK and BERG, 1982).

The pH dependence of dissociation and voluminosity is explained on the basis of MCP content of the micelles and the electrostatic repulsion and attraction between changes groups (WALSTRA and VLIET, 1986).

It is suggested that decreasing of pH was caused an increase in the rate of enzymatic reaction of renneting. The maximum rate was found 6.0 pH (HOOYDONK et al., 1986b).

The rennet coagulation time of milk is increased with pH in the range from 5.2 to 7.0 (ERNSTROM et al., 1974). Above pH 7.0, para-kappa-casein micelles become progressively more stable and do not aggregate, on the other hand below 5.2 pH, the measurement of renneting process complicated by the isoelectric aggregation of casein micelles (HOOYDONK et al., 1986b).

Only a few studies have been carried out to find the pH dependence of the enzymatic reaction in milk. The maximum rate of proteolysis of two peptides containing the chymosin-sensitive Phe-met bond (residues 98-111 and 98-112) were at pH 5.4 respectively. The results of Mehaia and Cheryan (1983) are indicated that the rate of aggregation was increased with pH in the range from 5.5 to 6.6 (HOOYDONK et al., 1986b).

The degree of conversion which was needed to initiate aggregation depends strongly on pH. At pH 5.6 the time of aggregation is at 30% conversion. At the original pH of milk this value is 70%.

The rate of enzymatic coagulation of milk is related inversely to pH and sensitive to changes in pH in the range of 6.5 to 7.0 pH. This effect is compounded, however, by alteration of calcium ion activity which was caused by changes in pH. If calcium held constant, CT increases with above and below the natural pH of milk (McMAHON et al., 1984).

The clotting time (CT) is decreased by decreasing in pH. For renneted milk a slight decrease in voluminosity of micelles is found with decreasing pH (ZOON et al., 1989). Decreasing the pH of milk causes an increase in the rate of enzymatic reaction, with a maximum on rate at pH 6.0. By decreasing in pH, aggregation of micelles starts at a lower conversion of kappa-casein and the rate of aggregation and gel formation increases, and it is found by HOOYDONK et al. (1986a) and ZOON et al., (1989) that the ultimate curd firmness was increased with decreasing pH.

The rate of coagulation increases with decreasing pH. When pH is decreased to 5.05, after 500 seconds coagulation of casein micelles reaches an enough portion but it could not shown change at pH 5.1 (BRINGE and KINSELLA, 1990).

MATERIALS AND METHOD

Reconstituted skim milk:

Low heat skim milk powder (300 g) was dissolved in 2500 ml of calcium chloride solutions (0.5, 1, 10, 50, 100 mM) at room temperature. They were used as Berridge Substrate.

Commercial calf rennet:

Calf rennet (90% chymosin+10% pepsin) with a strength of 1:10,000 (supplied from PEYMA İSTANBUL, TURKEY) was used with dilution of 1:10.

pH adjustment of the milk samples:

For some of the samples, pH values were adjusted to 6.55 pH at 4 °C by slowly addition of 1 mole/liter HCl or NaOH with vigorous stirring for calcium work. After overnight storage at refrigeration temperature (4-7 °C), all samples were heated to 30 minutes equilibration. For studying in pH, pH samples were adjusted to 5.40, 5.51, 5.56, 6.14, 6.32 and 6.51 pH.

Heat treatment:

One part of samples for calcium study were heated to 63 °C and kept 30 minutes at this temperature before overnight storage.

Normal samples preparation:

One part of samples were prepared with non adjustment of pH and non-heat treatment. These samples prepared with calcium chloride solutions at 0.5, 1, 10, 50, and 100 mM concentrations respectively and than kept in refrigerator for overnight. At they were heated to 30 °C and pH measured after 30 minutes equilibration.

Renneting of samples:

One milliliter of diluted (1:10 v/v) commercial rennet was added to milk sample (250 ml) in a beaker. At the time zero after stirring, 10 ml of renneted sample was pipetted into a test tube for determination of clotting time, it was run duplicated. And then cutting time was determined at residual samples in the beaker.

Determination of clotting time and renneting time:

The clotting time of all samples were determined by the method of Berridge (BERRIDGE, 1952). Clotting time is described as the time needed for visible clots. The time is needed for the formation of a film gel was described as renneting time. It was determined by feeling with a spatula for optimum gel cutting time.

Determination of time of syneresis (TS):

After gel firmness was reached to a maximum, gel cutted into 1 cc particles, and all of samples were put down into a filter paper covered funnel. The time needed for from cutting to collecting 50 ml of whey into the measuring cylinder was described as time of syneresis (TS). It was accepted as initial rate of syneresis.

RESULTS AND DISCUSSION

In this study, it is suggested that the calcium ion activity has an effect on enzymatic stage of renneting reaction moreover than dropped pH. Therefore after preparation of the samples, one part of them was adjusted to 6.55 pH and others non-adjusted, and than clotting time (CT) and renneting time (RT) was determined.

It is found that there was a considerable variation between calcium chloride concentration and pH with CT in non-adjusted samples. These results are in agreement with PAYENS (1979), HOOYDONK and WALSTRA (1987a, 1987b), BRINGE and KINSELLA (1986). The effect of calcium chloride concentration on CT and RT without pH adjustment are shown in Figure 1.

With respect to point of view of DALGLEISH (1983) it is obvious that the effect of calcium ions on the renneting process was more than obtained by GREEN (1982), as it was not a simple charge reduction of converted micelles. The way which was the simplest to produce this view, is determination of CT with increasing calcium concentration at adjusted-constant pH. Although the more detailed studies is continued by us.

The effect of increasing concentration of calcium chloride on the clotting time of pH-adjusted samples is shown in Figure 2.

As the effect of calcium on aggregation reaction is well known, it was not included detailed into this paper.

Also after heat treatment the effects of calcium content on CT and RT of none pH-adjusted samples were obtained. When Figure 3 are examined it is shown that decreasing calcium ion activity was caused by heat treatment, the effect of calcium chloride addition on enzymatic and flocculation reactions during the renneting was lowered. With this point of view it is also suggested that addition of calcium chloride to the cheese milk, which is a common treatment in cheese making, have to be treat after heat treatment agreement with WALSTRA and VLIET (1986), HOOYDONK and WALSTRA (1987a, 1987b), HOOYDONK et al., (1986c). As a result, the effect of calcium on the renneting process is not only increased the rate of proteolysis by decreasing pH or only effective on aggregation reaction as obtained by several authors. We are suggested that in agreement with DALGLEISH (1983) and GREEN and MARSHALL (1977), calcium has an effect, independent from pH, on the rate of proteolytic reaction in renneting process. Increasing calcium ion activity has a considerable effect on clotting time expect it's decreasing effect on pH.

As it shown in Figure 1 and Figure 2, calcium chloride concentration has an effect individually, despite of pH, on CT during the renneting process of milk. But there are two point of importance in this process. For pH adjusted samples 1 mM and 50 mM concentrations are shown as spoiling points for both of CT and RT.

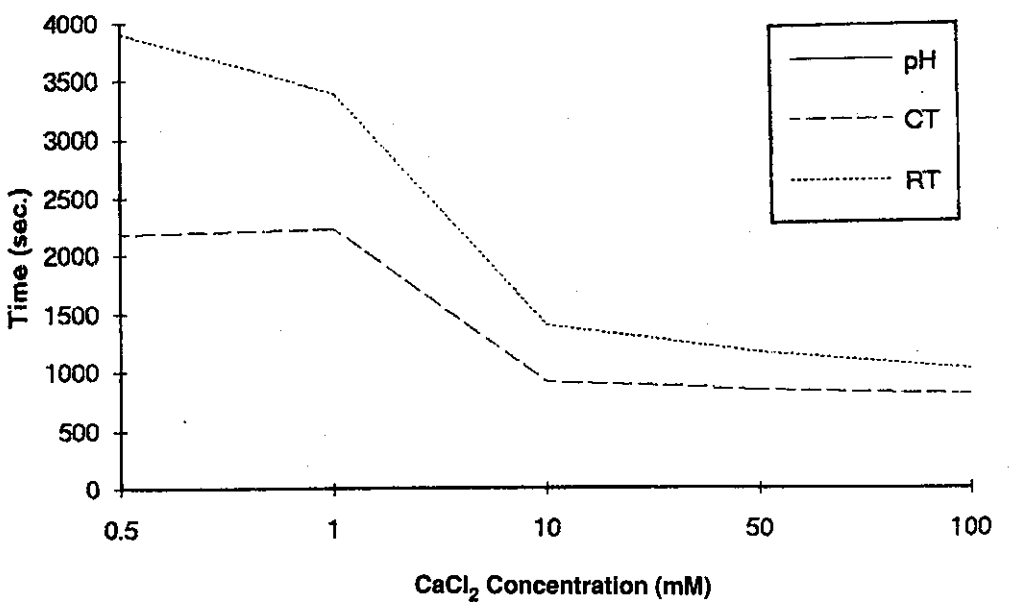

Fig 1. The effect of calcium chloride concentration on the CT and RT of non pH-adjusted samples.

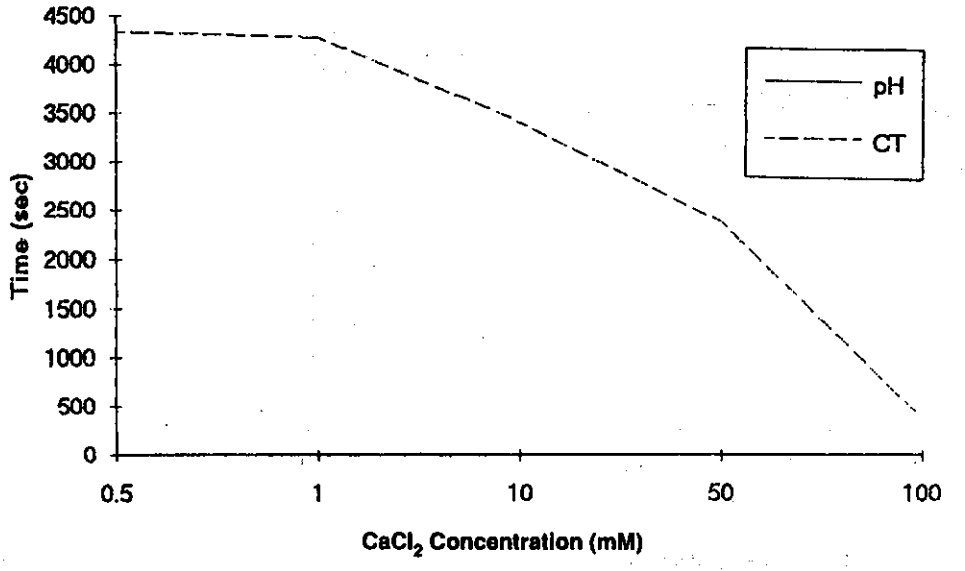

Fig. 2. The effect of calcium chloride concentration on the CT of pH-adjusted samples.

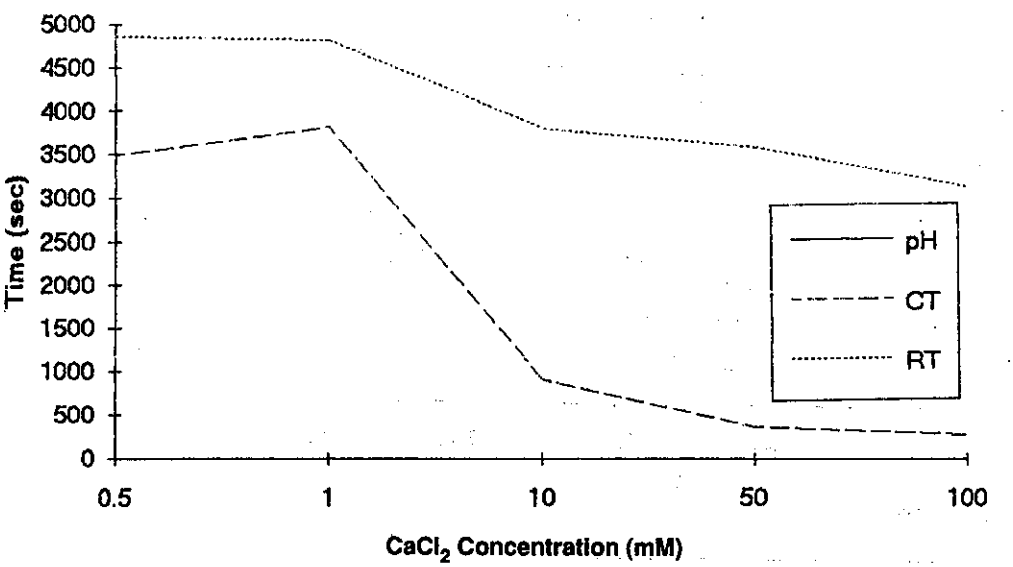

Fig. 3. The role of heat treatment (63°C/30 min) in the effect of calcium chloride concentration on CT and RT

The latter observation are agreement with Figure 3. But for heat-treated samples 1 mM, 10 mM, and 50 mM concentrations are appeared as spoiling points altogether.

It is also suggested that pH is a significant effect on enzymatic reaction as much as flocculation reaction during the renneting of milk. It was observed that clotting time (CT) of milk was effected by changing pH as much as renneting time (RT) and time of syneresis (TS). There is a logarithmic correlation between them, not linear. Effect of pH on CT, RT and TS are shown in Figure 4 and Figure 5 (double-logarithmic variation and linear variation respectively).

It is shown that RT is much more effected by pH alterations. However some of authors are suggested that pH was not an important factor for enzymatic stage of renneting. But as shown in Figures, there is a significant interaction between pH and CT. Figure 6 shows the effect of pH on CT with double-logarithmic regression plot and correlation. Although the correlation coefficient is not enough for suggesting, it is significant ($P<0.05$).

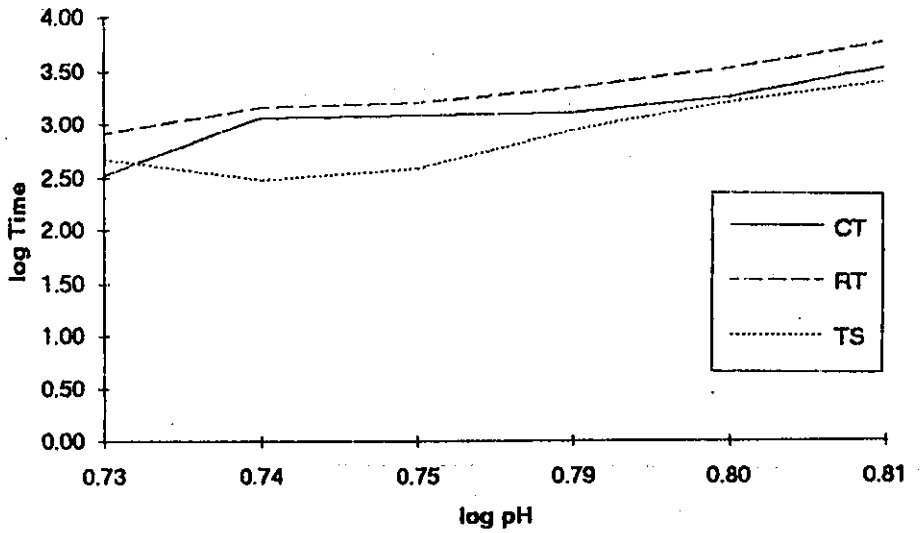

Fig. 4. Effect of pH on RT, CT and TS (double-log interactions)

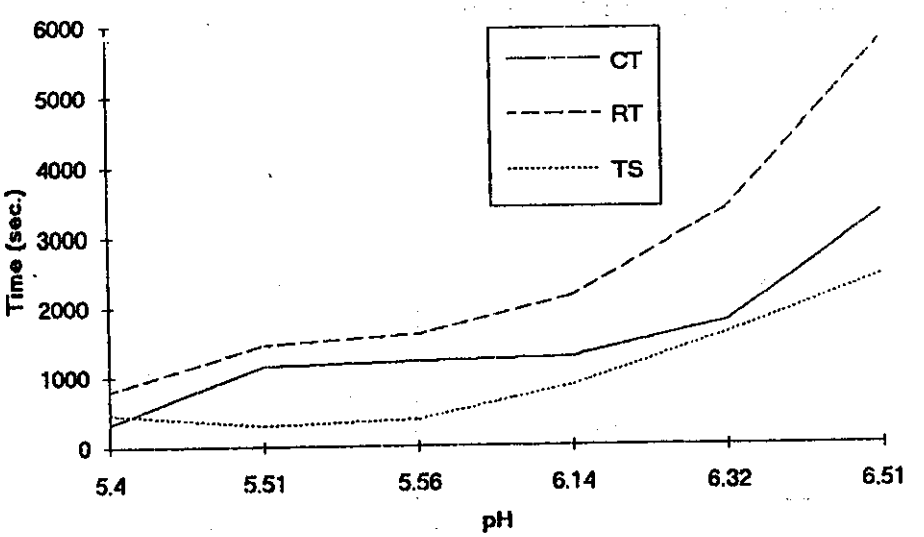

Fig. 5. Effect of pH on CT, RT and TS

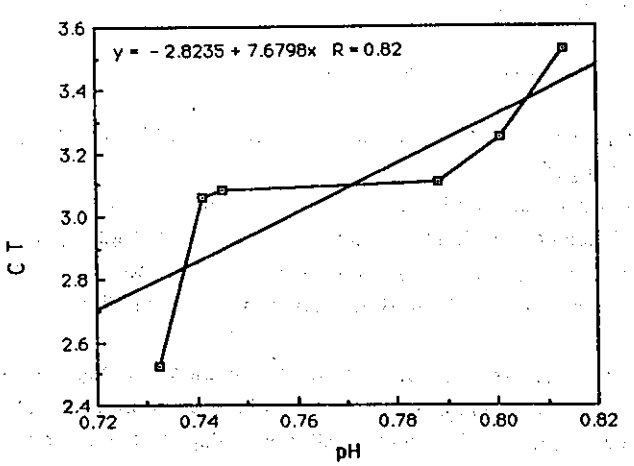

Fig. 6. Effect of pH on CT (double-log regression)

The relation between pH and RT is in agreement with some of authors (8; 2, 7; 5). The interaction of pH and RT with double-logarithmic regression is shown in Figure 7 ($p<0.01$)

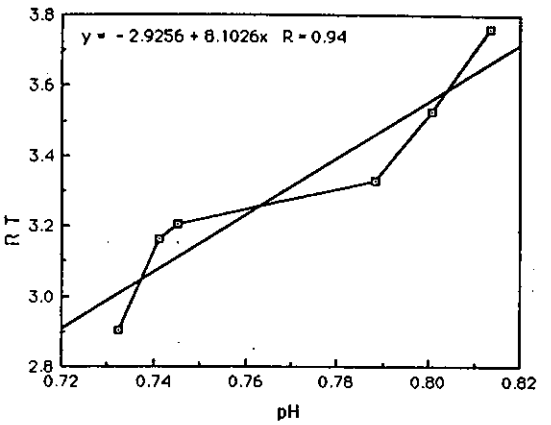

Fig. 7. Effect of pH on RT (double-log regression)

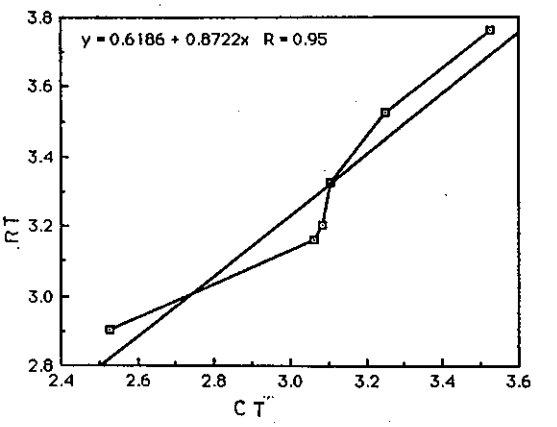

Fig. 7. Effect of pH CT-RT interaction (double-log regression)

The rate of regression at initial stages is effected by decreasing pH. This observation is in agreement with BIJGAART (1988). The efffect of pH on syneresis shown in Figure 4.

It is well known that there was a relationship between CT and RT, effected by pH, calcium ion activity, temperature, enzymatic activity etc. The effect of pH on this phenomenon is shown in Figure 8 ($p<0.01$). As a result pH rennet added milk effects the renneting process. Changes in CT, RT and TS at the pH range from 5.51 to 6.32 are drastic. Especially at pH 5.51 and 6.32, all of the parametric times have an increasing tendency, there were peak values at these pH. Below pH 5.51 there are a minimum value and above pH 6.32 there was maximum value.

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