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Research Paper

Stabilization of acidified milk drinks using pectin

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Abstract

The present study was carried out based on a special capacity of high methoxyl pectin in stabilizing acidified milk drinks. The presence of an optimal concentration of high methoxyl pectin can prevent the defects of acidified milk drinks such as protein flocculation, sedimentation and macroscopic wheying-off. A narrow range of pectin including 0, 0.1, 0.15, 0.2, 0.25, 0.3, and 0.4% (w/w) was added to acidified milk drink. All samples were subsequently heat treated at 70°C for 10 min, except for control samples in order to identify the optimal pectin concentration for stabilization of acidified milk drinks under processing conditions. The results showed that adding 0.15-0.4% pectin induced small particle size, low viscosity and limited sedimentation, thereby the acidified milk drinks were stable during 6 weeks of storage.

Keywords: Pectin, Acidified milk drinks, Stability, Casein micelle aggregation, Flocculation.

Introduction

Currently, acidified milk drinks (AMDs) have become popular amongst developing products, especially in the Asian market. This product is normally produced by diluting and homogenizing yoghurt or fermented skim milk gels and it is required to be stable, homogenous and not too thick. Unfortunately, the natural properties of milk proteins in acidified condition (pH around 4) are extensive casein micelle aggregation, sedimentation and subsequent macroscopic whey separation [1]. Many studies have illustrated high-methoxyl pectin (HMP) as an effective stabilizer in preventing these undesirable phenomena [2, 3, 4, 5].

The mechanism behind the stability of AMDs induced by pectin can be briefly assumed according to the following summaries:

- At pH around 4, casein micelles are supposed to have positive charges and pectin having the function of negatively charged polysaccharides will be absorbed onto the casein micelle surfaces as the result of an electrostatic attraction [6, 7].

- According to Tromp *et al*. [5], the absorption of pectin onto the casein micelle surfaces only occurs at the charged blocks; whereas the uncharged block chains protruding from the casein micelle surfaces as loops may cause a repulsive interaction between casein micelles. Thereby, the pectin can stabilize the individual protein particles in AMDs.

It is well known that the stabilization of AMDs is dependent upon many factors such as pH range, protein content, pectin concentration, etc. In this present work, the effects of pectin concentration and heat treatment on the stabilization of AMDs were emphasized.

Adding too little or too much pectin to AMDs can lead to the formation of an unstable system. If the amount of pectin added is not sufficient to cover all the casein micelles, bridging flocculation will occur and result in an extremely unstable system when compared to no pectin addition [6]. On the other hand, if the added pectin concentration is too high and exceeds full coverage of the particle surfaces, the AMDs will be unstable again. This phenomenon is explained as a result of depletion flocculation which separates the AMDs into concentrated phase and depleted colloidal phase [3, 8]. Moreover, Dickinson [9] and Maroziene *et al*., [3] have proposed that if the thickness of the concentrated layer further increases, the system may form a sufficient gel network in the aqueous solution.

Many studies have shown the narrow range of optimal pectin concentration could be used to stabilize the AMDs; e.g. 0.3% [5], 0.5% [8], 0.3-0.5% depending on the consumer demands [10], 0.2-0.4% and 0.6% with and without the effect of homogenization, respectively [11]. Considering these findings, the purpose of the present study is to investigate the influences of pectin on the stabilization of AMDs, as well as to determine the optimal pectin concentration for stabilization of AMDs.

Materials and Methods

Materials

All samples were prepared at pilot plant scale using commercial yoghurt with very low fat content of 0.1%. The protein content of yoghurt was 4.3%. Before producing AMDs, the pH of yoghurt was adjusted between 4 and 4.3 by 10% citric acid.

A 2% pectin solution (YP-115L, degree of esterification 72%, CP Kelco, Denmark) containing the proportion of 50% sugar was prepared by using the Silverson mixer to dissolve the pectin mixture in hot de-ionized water stirred at maximum speed. This solution was subsequently diluted with the proper proportions of de-ionized water to reach the following target pectin concentrations of 0, 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4%.

AMDs preparation

The yoghurt was stirred well with the diluted pectin solution in the vortex of Silverson mixer at maximum speed for 2.5 min. The samples were subsequently homogenized at the pressure of 150kPa/cm^2 for 5-10 min by Rannie homogenizer, MINI-LAB. Heat treatment samples were pasteurized at 70°C in the water bath for 10min and immediately cooled to room temperature in an ice bath. All samples then were stored in plastic containers at 5°C until further use.

Viscometry

Viscometry was performed by a Bohlin CVO rheometer. The temperature of 13°C and the interval shear rates of 1-300s^{-1} were the setting parameters for the measurements. The viscosity of samples considered as Newtonian behaviour was obtained from the slopes of the linear curve between shear rates and shear stress by applying a "LINEST" function of Microsoft Excel program.

Particle size measurements

Measuring the particle size distribution of the casein micelles was carried out on Matersizer (Malvern Instruments Ltd.). The given results were evaluated according to the Malvern presentation code of 5NQD, representing for a relative refractive index of particles of 0.01 and a refractive index of dispersant of 1.33. The stirring speed was halfway and obscuration reading was approximately in between 8-12%.

Sedimentation measurements

The formation of sedimentation occurring during storage was estimated by using Turbiscan MA 2000. The instrument combining a pulsed near infrared light source (λ = 850 nm) with a double detection, transmission and back scattering, vertically scans the whole height of test tubes and presents results in the graphics. The sedimentation intensity was evaluated from the changes of back scattering curves during storage.

Microscopy

Confocal Laser Scanning Microscopy (CSLM) was used to observe the overall microstructure of AMDs. From the given images, the average area size of each sample was obtained by using the image processing and analysis software of WCIF ImageJ. The image processing was carried out in the threshold of binary image and the noise from the system was reduced twice to improve the image quality. Fig.1 gives an example for observing the changes between the original image and the processed one.

Multivariate data analysis

Principle component analysis (PCA) with the transform of auto-scale combining full cross validation was applied by LatentiX (version 1.00) to investigate the correlations between variables and samples as well as to describe multivariate information of the data based on plots and graphics.

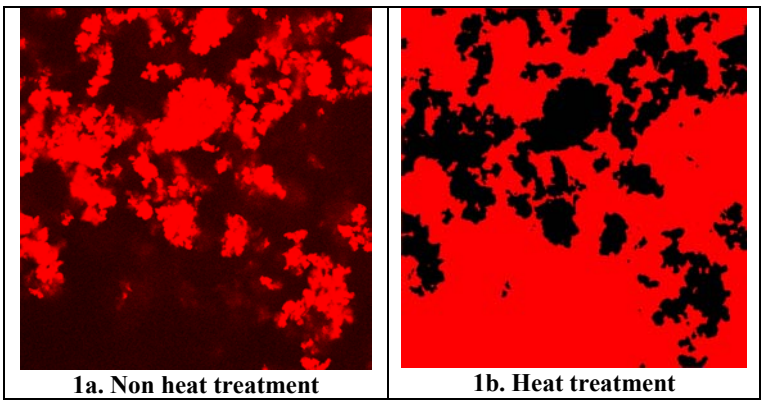
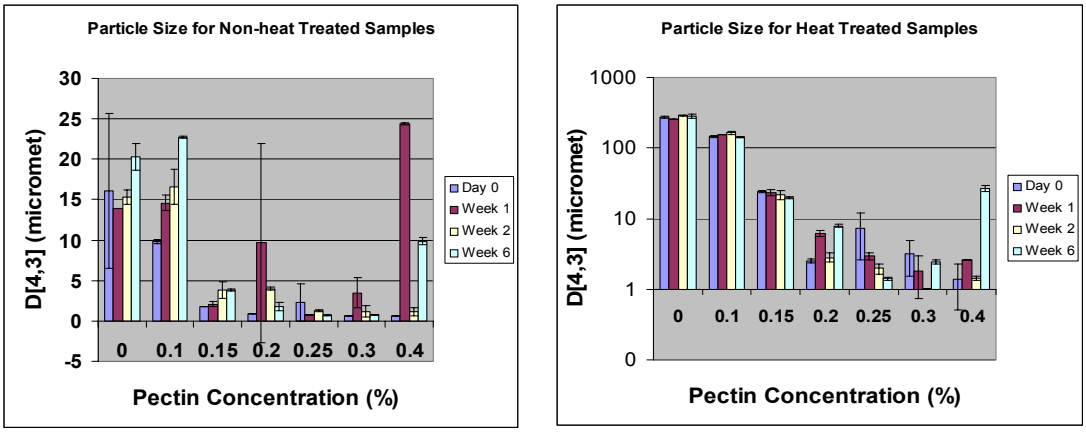


Figure 1. Confocal scanning laser images of AMDs without pectin and non-heat treatment before (1a) and after image processing (1b).

Results and Discussion

Particle size

As a result of the effective interaction between casein micelles and pectin, the volume moment mean of particle (D [4,3]) of samples added 0.15-0.4% pectin are much smaller than that of low pectin concentration and without pectin samples (Fig. 2). This result illustrates that casein micelles aggregated and resulted in the large particles at the sample without pectin. For this reason, the AMDs could not avoid being unstable if no pectin had been added. In addition, bridging flocculation clearly occurred when adding insufficient pectin (0.1%) to absorb on the casein micelle surfaces. This leads to the particle size of sample added 0.1% pectin was tendentiously larger than that of the sample with no addition of pectin (Fig. 2a). In contrast, the particles size of heat treated samples with no pectin was larger than that of samples with added 0.1% pectin (Fig. 2b). The contrary results indicate the obvious effect of heat treatment on the particle size.



2a. Non- heat treatment **2b. Heat treatment**

Figure 2. Changes in average particle size D [4, 3] during storage of non-heat treated (2a) and heat treated samples (2b).

It is clearly realized that heat treatment increased particle size because the particle sizes of heat treated samples are much larger (nearly 10 times) than that of non-heat treated samples at 0.1% pectin and without pectin (Fig. 2b). Furthermore, larger clusters of caseins were also found at higher pectin concentration of heat treated samples when compared to non-heat treated samples. This result is not surprising since it agrees well with the theories. Thermal treatment causes the whey protein to denature and some of this associates with casein micelles via hydrophobic interaction. This leads to accelerated protein aggregation and an increase in particle size [12, 13]. The results are furthermore similar to values reported by Sejersen *et al*. [1] who found larger casein aggregates at samples with low pectin concentration (≤0.1%) under microcopy. Sejersen *et al*. [1] have also proposed that the instability of AMDs after heat treatment derives from the de-sorption of outer pectin layer on casein micelle surfaces through the detectable decrease in zeta potential of casein aggregates after heat treatment.

The average area size of the particles from Confocal Laser Scanning Microscopy (CLSM) is defined as the particle size from Mastersizer. The given results from CLSM also gave the same tendency as that from Mastersizer. Without pectin and 0.1% pectin samples have larger average area size than the samples with a high amount of pectin (Fig. 3). However, a poor correlation between the results from CLSM and Mastersizer has been noticed (R^2 below 0.46, not shown) because of the high standard deviations of both measurements. They do, however, still have the same tendency (Fig. 2 & Fig. 3).

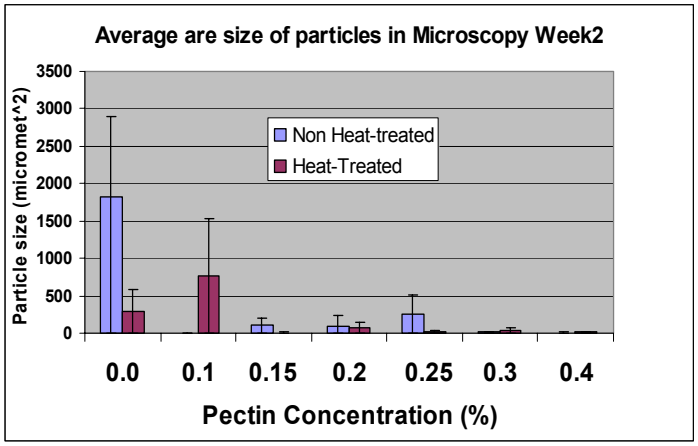
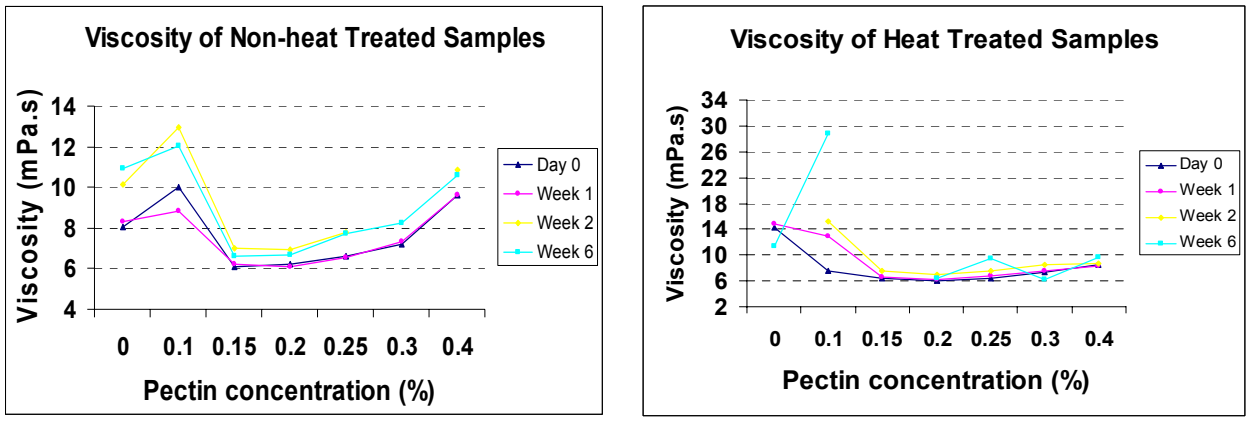


Figure 3. Plots of the average area size of particles in micrometer square at different pectin concentration and the storage time of week 2.

Viscosity

The viscosity of all samples is considered as Newtonian fluid and the results were obtained from the slopes of the linear curves between shear rates and shear tress. However, it should be noted that the samples having no pectin and low pectin concentration of 0.1% were less than Newtonian behaviour since the small regression values R^2 (below 0.5, not shown), whereas the rest of the samples fitted well with the Newtonian flow curve. It indicates that the AMDs are only homogenous and stable whenever sufficient pectin is added.

Plots of viscosity vs. pectin concentration (Fig. 4) show the viscosity was highest at 0.1% pectin samples and followed by the samples of 0% pectin in both heat treatment and non-heat treatment. The highest viscosity occurring at 0.1% pectin is due to bridging flocculation. When not adding enough pectin to cover all casein micelles, many of which together share a pectin chain, this leads to greater casein aggregation and influences the viscosity [3, 4]. In contrast, the viscosity of samples 0.15-0.3% added pectin was low and stable as a result of good pectin-protein interaction. However, by further increasing pectin level to 0.4%, the viscosity increased again because of the depletion flocculation and an increase in the amount of free non-absorbed pectin in the serum [4]. From the results of viscosity, it may be assumed that good stability can be reached at an optimal pectin concentration at the point of minimum viscosity.



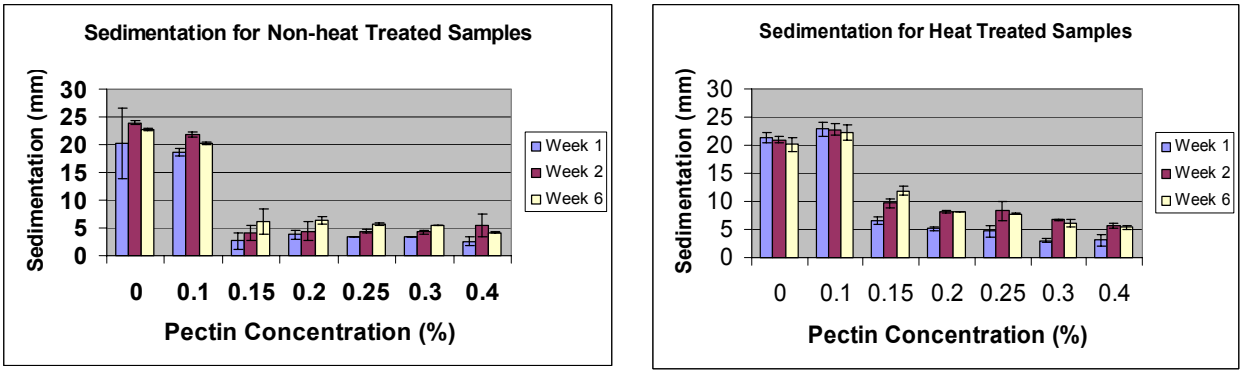
4a. Non- heat treatment **4b. Heat treatment**

Figure 4. Changes in viscosity of non-heat treated samples (4a) and heat treated samples (4b) as a function of pectin concentration.

Sedimentation

The sedimentation was estimated based on the changes in back scattering curves during storage. Plots of the raw data for each sample back scattering vs. the length of sample in millimetres were constructed (not shown). The back scattering curves of each sample at day 0 were defined as the basic lines and they were thereafter crossed by the back scattering curves at different storage time of week 1, 2 and 6. Thereby, the sedimentation lengths at different storage time were determined as the distances from the bottom of test tube until the points where the back scattering curves crossed the basic curves.

Agreeing well with the given results from particle size measurements and viscometry, the sedimentation tendentiously occurred at samples having large particle size and high viscosity. Fig. 5 shows that adding 0.15-0.4% pectin reduced the formation of sedimentation in both heat treated and non-heat treated samples, when compared to without pectin samples and insufficient pectin samples. It is well known that without stabilizer or not adding enough pectin cannot prevent casein micelle aggregation that is subject to phase separation and therefore the formation of sedimentation is indispensable [6]. Moreover, the sedimentation also increased over time, since the casein particles need time to form with the sediment.



5a. Non- heat treatment **5b. Heat treatment**

Figure 5. Sedimentation represented in millimetres at different pectin concentration of non-heat treated samples (5a) and heat treated samples (5b).

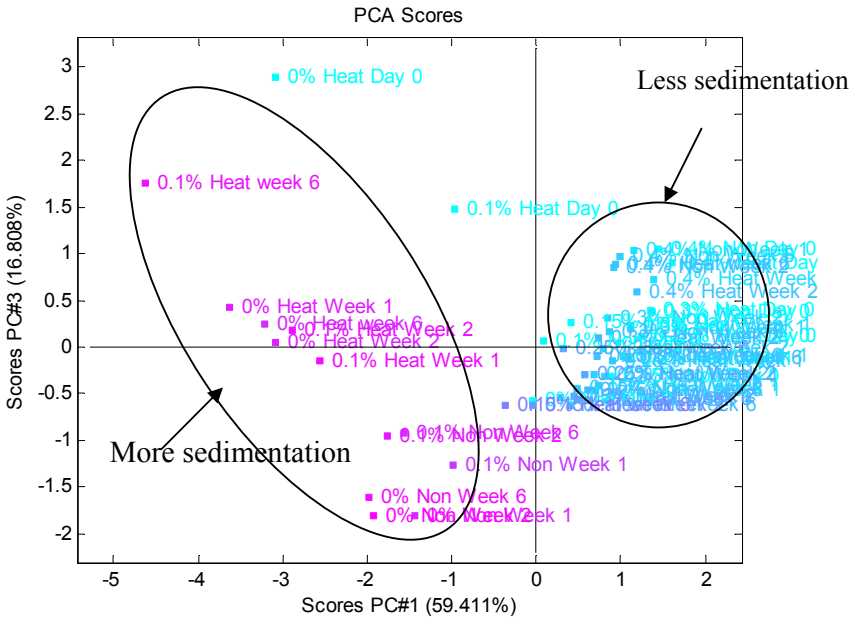


Figure 6. Principle component analysis score plot (PC1 vs. PC3) of the sedimentation data of all samples.

A question could occur that any difference can be seen within the high pectin concentration group, i.e., between the heat treated and non-heat treated samples are they different in terms of sedimentation? It is difficult to answer from the bar graphs as Fig. 5 and a multivariate data analysis was applied to support an answer to this question. PCA score plot (PC1 vs. PC3) with high percentage of explanation (more than 76%) clearly separates all samples into two groups (Fig. 6). The high pectin concentration group located on the right correlates to short sedimentation length and the remaining group on the left correlates to long sedimentation length. In addition, no difference can be seen among samples 0.15-0.4% added pectin between heat treatment and non-heat treatment.

Multivariate data analysis

PCA bi-plot combining different variables and samples in one plot is plotted to observe the correlations between variables and samples as well as within variables. Fig. 7 obtained from the PCA bi-plot (PC1 vs. PC3) with the percentage of variant explanation of approximately 77% shows that a group of samples with more than 0.1% added pectin negatively correlate to the variables of particle size, viscosity and sedimentation. On the other hand, no addition of pectin or adding insufficient pectin samples which induced bridging flocculation, positively correlates to large particle size, high viscosity and long sedimentation length. The given results strongly demonstrate and strengthen the theories of the mechanisms behind the stabilization of AMDs induced by pectin. The AMDs evidently cannot become a good stabilized system unless an appropriate amount of pectin is added [3, 5].

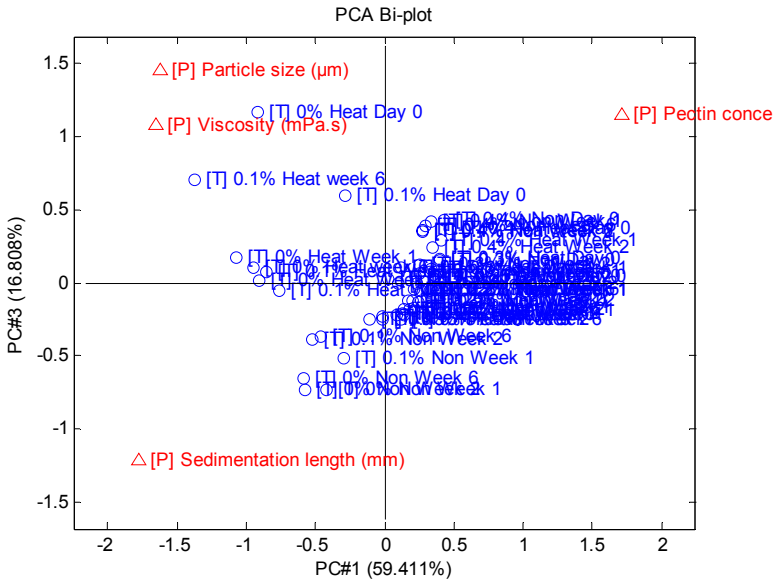


Figure 7. PCA bi-plot shows the negative correlation between pectin concentration and the variables of particle size, viscosity and sedimentation causing the instability of AMDs.

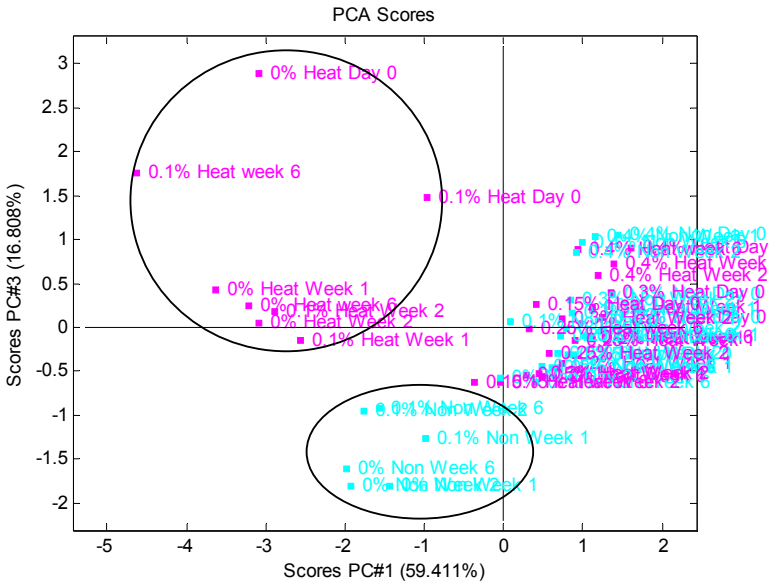


Figure 8. PCA score plot (PC1 vs. PC3) illustrates a clear difference between non-heat treated samples and heat treated samples at 0% and 0.1% of pectin.

A clear difference between non-heat treated and heat treated samples with 0% and 0.1% pectin can be observed in the PCA score plot of PC1 vs. PC3 (Fig. 8). The heat treated samples are located in the upper part, whereas the non-heat treated ones are separately located in the lower part. This indicates that thermal treatment actually affects the stabilization of AMDs, particularly in terms of particle size (Fig. 2). This result agrees well with the results obtained by Sejersen *et al*. [1] who have found the heat treated samples with no pectin and 0.1% pectin are more unstable than the non-heat treated ones. The given results can be explained by the decrease in zeta potential of casein aggregates in AMDs that derive from the partial de-sorption or re-arrangement of pectin after heat treatment [1]. Therefore, thermal treatment influences the stabilization of AMDs. Contrary to the given results at 0% and 0.1% added pectin samples, no difference can be observed between the heat treated samples and non-heat treated samples from 0.15-0.4% added pectin.

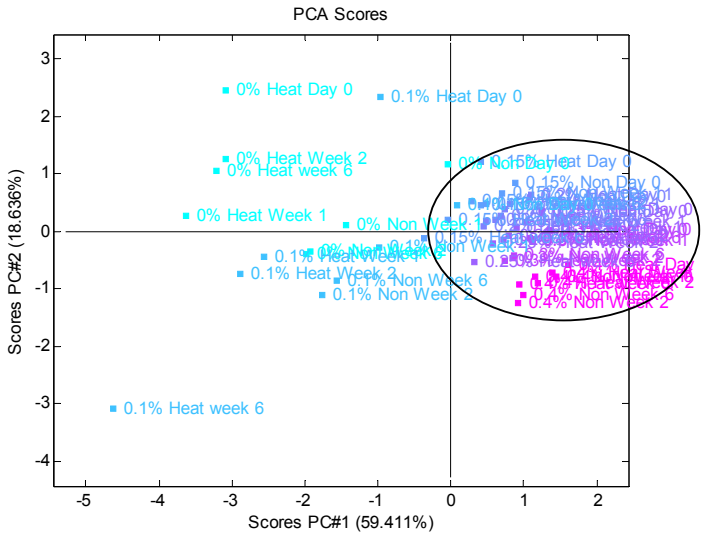


Figure 9. PCA score plot of two first components (PC1, PC2) shows the group formation of samples added from 0.15-0.4% and this group is far away from the others.

The present study has the aim of identifying an optimal pectin concentration that should be used to create good stabilization of AMDs. However, the PCA score plot (PC1 vs. PC2) in Fig. 9 indicates the samples added from 0.15-0.4% pectin group together and they are different far away from the rest samples of 0% and 0.1% pectin. That means no difference can be seen within high pectin concentration samples. Therefore which concentration is the best in stabilizing the AMDs can not be concluded but a narrow range of 0.15-0.4% pectin could apply for the stabilization of AMDs in both heat treatment and non-heat treatment.

Conclusion

In the production of acidified milk drinks, it is possible to keep the system stable by adding an appropriate amount of high methoxyl pectin. No addition of pectin, or adding insufficient pectin, resulted in lager particle size, high viscosity and sedimentation which induced unstable AMDs. Heat treatment is an indispensable process in order to maintain the shelf-life of the products, however heat treatment accelerates the AMDs becoming more unstable, especially in the products with no pectin or low pectin concentration. While the optimal concentration that should apply cannot be concluded, a narrow range of 0.15%-0.4% pectin would be recommended for the stabilization of AMDs based on a small particle size, low viscosity, limited sedimentation and minimal effects of heat treatment.

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