Research on Turbidity and Surface Hydrophobicity of Milk Protein at Different Conditions

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Abstract. This study is mainly about determination of turbidity and surface hydrophobicity at different conditions. We use skim milk powder (SMP) as raw materials, the protein dispersion was prepared by dropping 1 wt% (protein basis) with the skim milk powder in Millipore water, and stirring at room temperature for 2 h. The samples were adjusted to different pH values (6.2, 6.3, 6.4 and 6.7) by stepwise addition of 0.1 mol/L HCl, then transferred to glass tubes and heated for 0-30 min at 80-100°C in a water bath. After the heat-treatment, the milk samples were immediately cooled to room temperature by immersion in an ice bath, and determining turbidity and surface hydrophobicity of milk protein at different conditions.

1. Introduction
Protein as raw material or additive is widely used in food, because it can improve the nutritional value and functional characteristics of food, in which the functional properties of protein is more important than the nutritional value [1,2]. Heat treatment can improve the functional properties of milk protein, and can reduce the number of microorganisms, prolong the shelf life of food, in addition, heat treatment can also achieve the expected properties of food, just like can increase the viscosity of yogurt [3]. In recent years, the dairy industry in China has achieved unprecedented development, especially the development of yogurt products. Relevant statistics show that in recent years, the production of yogurt in China is developing at a rate of more than 30%, and has become an indispensable important product in the life consumption of Chinese people [4]. The international demand for yogurt is also the same, showing an increasing trend year by year. It can be seen that yogurt, as a representative product of functional dairy products, has been widely accepted by people. With the increasing demand of people's living standard, the quality of yoghurt has become an urgent problem to be solved in the production of yoghurt. Factors affecting the quality of yogurt in the actual production has a lot of, such as heat treatment temperature [5-7], homogeneous pressure, the strain of external conditions such as inoculation, fermentation temperature, solid content [8], milk protein content and the influence of the internal factors such as the composition is very important also, in recent years, for milk protein affect yogurt gel has drawn the attention of some scholars. In fact, the reaction speed of milk protein has a very important effect on the quality of yogurt gel. The milk protein reaction rate is ultimately the rate at which casein and whey proteins are cross-linked to produce a polymer, and the formation of a polymer is bound to cause changes in properties such as turbidity, disulfide bonds, and surface hydrophobicity of the protein [9]. There are a large number of trials show that heat treatment can lead to breast different forms and levels of physical and chemical properties change, such as milk protein denaturation, fat oxidation, the loss of lactose, for the destruction of vitamins and Maillard reaction,
etc., at the same time of dairy nutrition loss also produce also some toxic substances, such as chaff amino acid and the Maillard reaction product [10]. The effects of heat treatment on the physicochemical properties and structural changes of milk are summarized as follows. The interaction between casein and whey protein can be significantly affected by a slight change in pH during heat treatment. When the pH decreased from 6.9 to 6.35 at 90°C for heat treatment, the interaction between casein and whey protein was shown in figure 1-1. At higher pH, the whey protein on the surface of casein micelle was evenly distributed and a large number of whey proteins were aggregated. At pH 6.55, there was no whey protein aggregation in the whey phase. At lower pH, clusters of whey proteins bind to the casein micelles and attach to the surface of the casein micelles [11].

![Figure 1. Effect of pH on milk protein interaction after 25 min heat treatment at 90°C](image)

In this research, skimmed milk powder (SMP) was used as raw material to study the changes of the turbidity and surface hydrophobicity of milk protein solution under different conditions (temperature, heat treatment time, pH) by heat treatment method.

2. Materials and methods

2.1. Materials
Skimmed Milk Powder (SMP) were purchased from New Zealand Fonterra Group, the content of protein was 34.67 wt%, casein 27.92 wt% and whey protein 6.75 wt% by Kjeldahl method (Kjeldhal analysis: N×6.38. Disodium hydrogen phosphate (Tianjin Institute of Guangfu Fine Chemicals), Sodium dihydrogen phosphate (Tianda Chemical Reagent Factory, Dongli District, Tianjin), Hydrochloric acid (Tianjin Yaohua Chemical Reagent Co. Ltd), Ice acetic acid (Tianjin kaitong chemical reagent co., LTD), ANS fluorescent probe The Sigma company).

2.2 Instruments for experiments
Model AL204 analytical balance (Mettler Toledo Instrument (Shanghai) Co., Ltd), Electric heating constant temperature blast drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd), DK - 98 - II type A constant temperature water bath pot (Tianjin Tester Instrument Co. Ltd), PHS-3C precision pH meter (Mettler Toledo Instrument (Shanghai) Co., Ltd), UV-240IPC UV-visible spectrophotometer (Shimadzu Corporation of Japan), F-4500 fluorescence spectrophotometer (Hitachi Co. Ltd).

2.3. Methods

2.3.1. Measurement of turbidity. Referring to the method of Jean et al. [7], the heat-treated SMP solution was diluted to a certain concentration with deionized water (OD<1.5), and the OD value was measured by a spectrophotometer at a wavelength of 600 nm with deionized water as blank zero setting.

2.3.2. Measurement of surface hydrophobicity. The surface hydrophobicity of milk protein was determined by referring to the relevant ANS fluorescence probe method and improved [12]. Note: ANS is 8-phenylamino-1-phenol iodide, which is a kind of anionic hydrophobic fluorescent needle. It has good fluorescence in non-polar environment. The heat-treated SMP solution was diluted with 0.01 mol/L phosphoric acid buffer (pH 6.7) into a series of samples with protein concentrations of 0.4 wt%, 0.2 wt%, 0.1 wt%, and 0.05 wt%, respectively. Taking 4 mL diluted solution of SMP and adding
20 μL ANS (8 mmol/L, dissolved in 0.01 mol/L phosphate buffer, pH 6.7) fluorescent probes, blending, shelter from light at room temperature for 15 min, then perform a colorimetric test under a fluorescence spectrophotometer, excitation wavelength of 390 nm, emission wavelength of 470 nm and slit under the condition of 5 nm, determination of the relative fluorescence intensity of ANS binders. Then the protein solution concentration was plotted with the fluorescence intensity value, and the slope of the regression line with good linear relationship was taken as the hydrophobicity index of the protein surface. Note: When measuring surface hydrophobicity of SMP solution under different pH conditions, the buffer solution with the same pH should be selected.

3. Results

3.1. Changes of milk protein turbidity under different conditions.

3.1.1. The effect of pH on turbidity. The turbidity of the protein is measured by spectrophotometer. When the turbidity value of the solution changes, it indicates that there is polymer formation or dissociation. After dairy products are thermally denatured under different conditions, the hydrophobic interactions, sulphydryl interactions, and electrostatic forces between protein molecules are different, which proves that the degree of polymerization of casein micelles and whey protein is different, resulting in pores or particle size of protein polymer formed is different. The absorbance value measured by photometer is used to represent turbidity. The larger the absorbance value is, the smaller the pore or the larger the particle size in the sample to be measured, resulting in the lower the light transmittance. Therefore, the turbidity of SMP solution under different conditions was measured, and the changes of turbidity under different conditions were analyzed and compared.

In order to verify the heat treatment when the pH influence on protein aggregation, on the premise of not change the composition of milk protein, using dried skim milk SMP as raw materials, different pH of milk protein was studied the effect of turbidity in the polymer formation process, determine the test conditions for: different pH (6.1, 6.2, 6.3, 6.4, 6.7), protein content is 1% of the SMP solution, heat treatment under 90 °C 25 min turbidity changes the results as shown in figure 2.

![Figure 2](image_url)

**Figure 2.** Variation of optical density at 600 nm of milk solution by heating 1 wt% (protein basis) of SMP solution with different pH values at 90 °C for 25min.

As can be seen from the above figure, slightly changing the pH of the solution plays an important role in the polymerization between the whey protein and casein. As the pH decreases, the turbidity value increases, and the turbidity value increases from the initial 0.27±0.016 to 0.68±0.019.

3.2.2. Effect of heat treatment time on turbidity. The time of heat treatment can affect the denaturation degree of milk protein, so the time of heat treatment may also have some influence on the formation of protein polymer. On the premise of not change the composition of milk protein, we take the dried skim milk SMP as raw material, study the different heat treatment time on the protein turbidity in the
process of polymer formation, the influence of test conditions defined as: the SMP solution pH6.7, protein content is 1%, at 90 ℃ under different heat treatment (0, 5, 10, 15, 20, 25 and 30 min) the change of the turbidity of the results as shown in figure 3.

![Figure 3](image)

**Figure 3.** Variation of optical density at 600 nm of milk solution by heating 1 wt% (protein basis) of SMP solution with pH 6.7 at 90 ℃ for different heating time.

It can be seen from the figure above that the turbidity value of SMP solution tends to increase with the increase of heat treatment time, indicating that polymer is formed with the increase of heating time, in which the turbidity value rises from the initial 0.297±0.003 to 0.395±0.003.

3.2.3 The effect of temperature on turbidity. The test conditions were determined as follows: pH6.7, protein concentration of 1% SMP solution, heat treatment at different temperatures (80, 85, 90, 95 and 100 ℃) for 25min, turbidity change results, as shown in figure 4.

![Figure 4](image)

**Figure 4.** Variation of optical density at 600 nm of milk solution by heating 1 wt% (protein basis) of SMP solution with pH 6.7 at different heating temperature for 25 min

It can be seen from the above figure that the turbidity value of SMP solution tends to increase with the increase of heating temperature. The turbidity value rises from the initial 0.295±0.015 to 0.605±0.013. When the heating temperature exceeds 95 ℃, the turbidity value basically remains unchanged.
3.3 Changes of surface hydrophobicity of milk protein under different conditions

Heating is a necessary condition for the formation of protein polymers. Protein molecules usually show a tightly coiled structure. Heating makes protein molecules unfold, and the hydrophobic groups originally embedded in the curl are exposed to the outside, so that the hydrophilic groups originally outside the curl structure are relatively reduced. When heated to a certain temperature, all of the hydrophobic groups are exposed to the surface of the protein molecules that form the polymer. Even if the polymer is again exposed to a low temperature, the hydrophobic groups are present, but this is not true of all proteins. Therefore, the hydrophobicity of milk under different heat treatment conditions was determined to reflect the extent of protein extension during heat treatment, and the changes of surface structure of reticular polymers were analyzed and compared. After a certain time of heating and pH treatment, casein micelle and whey protein denatured in different degrees, and their molecular structure changes, in which the polymerization of casein micelle and whey protein or the dissociation of casein micelle itself will cause the change of the sample surface hydrophobicity. Therefore, it is of great significance to analyze the changes of the molecular surface structure of milk protein and the formation of protein polymers by studying the changes of the surface hydrophobicity of milk protein when pH, time and temperature are changed.

3.3.1. Effect of pH on protein surface hydrophobicity.

The test conditions were determined as follows: the change results of surface hydrophobicity under different pH (6.1, 6.2, 6.3, 6.4, 6.7) and heat treatment at 90°C for 25min were shown in figure 5.

![Figure 5. Variation of the surface hydrophobicity of milk protein by heating 1 wt% (protein basis) of SMP solution with different pH values at 90°C for 25 min.](image)

For SMP solutions (1% protein concentration) with different pH, pH has a certain influence on the surface hydrophobicity under 90°C heat treatment conditions. The test results are shown in figure 5. With the decreasing of pH, the surface hydrophobicity of proteins in SMP solution increased after heat treatment, and the surface hydrophobicity index was 1875.75±8.767, 1924.95±4.1, 2083.467±14.711, 2194.42±7.089 and 2198.3±12.6, respectively.

3.3.2. Effect of heat treatment time on protein surface hydrophobicity.

Heating time can affect the denaturation degree of milk protein, so the heat treatment time may also have some influence on the formation of protein polymer. The test conditions were determined as follows: the surface hydrophobicity of SMP solution with pH6.7 and 1% protein concentration was changed by different heat treatments (0, 5, 10, 15, 20, 25 and 30 min) at 90°C, as shown in figure 6.
Figure 6. Variation of the surface hydrophobicity of milk protein by heating 1 wt% (protein basis) of SMP solution with pH 6.7 at 90 °C for different heating time.

For the SMP solution (1wt% protein concentration) with pH 6.7, the heat treatment time has a certain effect on its surface hydrophobicity at 90°C. The test results are shown in figure 6. From the above results, it can be seen that the surface hydrophobicity of protein gradually increases with the increase of heat treatment time after the SMP solution. When heated for 30 min, the surface hydrophobicity of the protein at pH 6.7 was 2380.667±15.26. The surface hydrophobicity of the protein increased by 540±15.26 at pH 6.7 after 0 min heat treatment.

3.3.3 Influence of temperature on protein surface hydrophobicity. The test conditions were determined as follows: the surface hydrophobicity of SMP solution with pH 6.7 and 1% protein concentration was heat treated at different temperatures (80, 85, 90, 95 and 100 °C) for 25 min, as shown in figure 7.

Figure 7. Variation of the surface hydrophobicity of milk solution by heating 1 wt% (protein basis) of SMP solution with pH 6.7 at different heating temperature for 25 min

The surface hydrophobicity of skim milk powder protein is closely related to the heat treatment temperature. The test results are shown in the figure above. With the increase of heating temperature, the surface hydrophobicity of the protein increased, and the surface hydrophobicity index of the protein was 1677.733 ± 10.09, 1712.1 ± 12.67, 1841.1 ± 7.4, 1869.67 ± 16.16 and 2070.167 ± 28.69,
respectively. Taking the heat treatment temperature of 80°C as a reference, the surface hydrophobicity gradually increases with the increase of heat treatment temperature, increasing by 34.367, 163.37, 191.937 and 392.433, respectively.

4. Conclusion

4.1 Changes in turbidity
With the increase of heating temperature, the turbidity of SMP solution increased from 0.295±0.015 to 0.605±0.013. When the heating temperature exceeded 95°C, the turbidity remained basically unchanged. With the increase of heating time, the turbidity value of SMP solution tended to increase, indicating that with the increase of heat treatment time, the formation of polymer, turbidity value from the initial 0.297±0.003 to 0.395±0.003.; As the pH decreased, the turbidity value increased, and the turbidity value increased from 0.27±0.016 to 0.68±0.019.

4.2 Changes in protein surface hydrophobicity
With the decrease of pH, the surface hydrophobicity of proteins in SMP solution increased after heating, and the surface hydrophobicity index was 1875.75±8.767, 1924.95±4.1, 2083.467±14.711, 2194.42±7.089 and 2198.3±12.6, respectively. After heat treatment, the hydrophobicity of SMP protein surface increased gradually with the increase of heat treatment time. When heated for 30min, the surface hydrophobicity of protein at pH6.7 was 2380.667±15.26. The surface hydrophobicity of protein at pH6.7 was increased by 540±15.26 using 0min heat treatment as reference. With the increase of heating temperature, the surface hydrophobicity of the protein increased, and the surface hydrophobicity index of the protein was 1677.733±10.09, 1712.1±12.67, 1841.1±7.4, 1869.67±16.16 and 2070.167±28.69, respectively. Taking the heat treatment temperature of 80°C as a reference, the surface hydrophobicity gradually increased with the increasing of heating temperature, increasing by 34.367, 163.37, 191.937 and 392.433, respectively.

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