

Analysis on the effect of the various factors on immunoglobulin G in goat colostrum

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Abstract. The present study aimed to determinate the content of immunoglobulin G (IgG) in goat colostrum and the effected factors were discussed by single radial immunodiffusion. The results showed that the highest level of IgG was detected in the first day after partum, and decreased quickly over the length of the lactation period. No significant effect on IgG was seen with different ways of thawing and the different freezing time. IgG was almost completely lost at 85°C for 2 min. The maximum value of IgG was observed at pH 6.5, and it reduced significantly when the pressure was higher than 500MPa, and decreased with the increase of the concentrations of citric acid and Ca²⁺.

1 Introduction

As a kind of potential alternative of human milk source, goat milk is one of the most important nutritional food in the world, playing an important role in human daily life [1]. Goat milk is of high levels of biologically active proteins, such as immunoglobulin, lactoferrin, EGF, SOD. The immunoglobulins and oligosaccharides in the milk of humans and other mammals provide a significant amount of protection to the recipient.

Numerous factors, such as lactation period, parity, nutrition and genetic selection are known to impact colostrum immunoglobulin concentration [2]. IgG concentration values were reported only in a few studies conducted on a few animals from one breed [3, 4]. The immune quality of the colostrum in goat milk has been poorly explored and variation factors have neither been evaluated. So the objective of this study was to determine the IgG concentration of colostrum during the first seven days postpartum and to evaluate the effects of freezing time, different thawing methods, temperature, and different pH values, high-pressure processing, citric acid concentration, and calcium ion concentration on the concentration and activity of IgG in goat colostrum in order to provide theoretical references for industry in the utilization of goat colostrum.

2 Materials and methods

2.1 Collection and preparation of goat milk

Thirty samples goats were randomly selected from the Three Xi Goat Farm in Tai'an City (N35°52', E116°50'),

Shandong province, China. The colostrum samples (n=7*30) were collected from the 1st to 7th lactation day after parturition (7 d of lactation and once a day). Each sample was divided into several aliquots and transported to lab by cold chain.

2.2 Single radial immunodiffusion

The active IgG concentration was determined by single radial immunodiffusion according to Hadorn et al with some modifications [5]. A series of agarose plates were prepared in which the rabbit anti-goat antiserum was diluted 1:2, 1:4, 1:8, 1:16, and 1:32 (Sigma-Aldrich, St. Louis, MO, USA). Then, commercial standard goat IgG (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was dissolved in PBS to a series of standard solutions (40, 20, 10, 5, and 2.5 mg/100 mL) on every immunodiffusion well in the agarose to generate a standard curve, as shown in Figure 1. Subsequently goat milk colostrum were treated by the different thawing temperature, heating temperature, pH, and the effect of various factors on IgG were analyzed respectively.

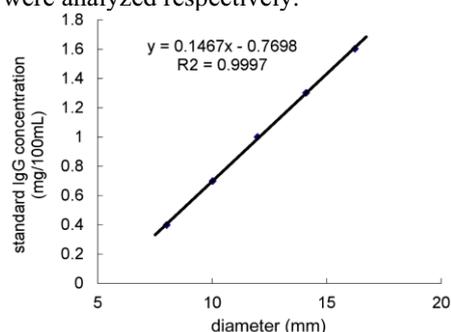


Figure 1. The logarithmic plot of standard goat IgG concentration

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2.3 Statistical analysis

All data were presented as means of at least three independent determinations \pm standard deviation (SD). Statistical evaluation was performed by using one-way analysis of variance (ANOVA) of the SPSS 19.0 Statistics program and Fisher's test with a significance level of $P < 0.05$ (in small letter) and very significance level of $P < 0.01$ (in capital letter).

3 Results and discussion

3.1 Changes of IgG from partum to 7 days postpartum

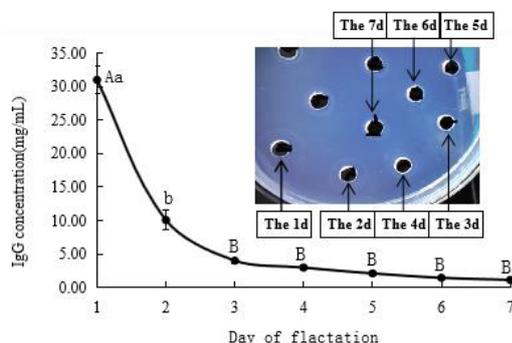


Figure 2. IgG change from partum to 7days postpartum

The concentration of IgG in goat colostrum were observed from 1 to 7 d postpartum, decreased significantly with prolonged lactation (Figure 2). IgG concentration in milking from 1 to 2 day was significantly higher than that measured from other days of milking during the total 7 days. The results are similar to those previous reported that the IgG content of the milk in the first 7 days after the milk production [6].

3.2 Analysis on the effect of storage time and thawing method on IgG

As shown in Figure 3, the IgG concentrations showed a slow decline with the increasing of the freezing time for goat colostrum. Compared with the first 45-day preservation, there was significant difference for the 60-day and extremely significant difference for the 75-day and 90-day. In conclusion, after 3 months of preservation at refrigeration temperatures of -20°C , IgG levels diminished by 10.42%, and the colostrum was still usable for new-born kids, although where possible it would be desirable to store colostrum for no longer than 45 days after collection. This was consistent with the finding that effects were observed within storage room at temperature of 4°C for 3 months, and try not more than a month as much as possible [7].

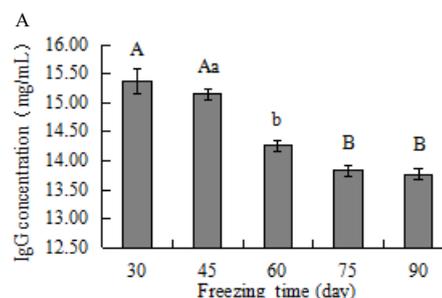


Figure 3. Changes of IgG with freezing time on

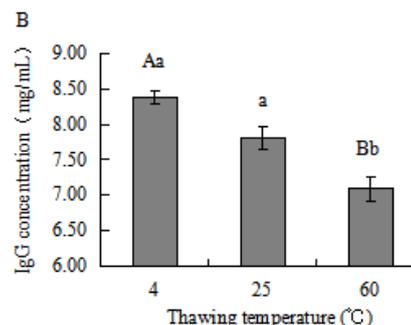


Figure 4. Changes of IgG with the thawing temperature

Figure 4 showed that the IgG concentration in goat colostrum thawed in 60°C water bath was significantly lower than that thawed at 25°C and 4°C ($p < 0.05$), but no significantly difference between 25°C and at 4°C . Argüello et al. ascertained that different thawing methods: refrigeration (4°C), room temperature (27°C), microwave (55°C) and hot water (60°C) made no difference on IgG concentration. But, repeated freezing and thawing can reduce IgG concentration [7]. It is generally known that thawing at 4°C takes time, so the room temperature (25°C) was the preferred thawing method for the freezing goat milk.

3.4 Analysis on the effect of heating temperature on IgG

As Figure 5 showed, the concentration of IgG has a significant decline as temperature was increased. When the goat colostrum was heated at 65°C , the concentration of IgG was found to be invariable, in general, regardless of treatment duration. The trends are in agreement with those of McMartin et al. with conditions of 63°C for 120 min and of Tyler et al. with conditions of 63°C for 30 min, IgG was still stable, used for pasteurization processing [8, 9].

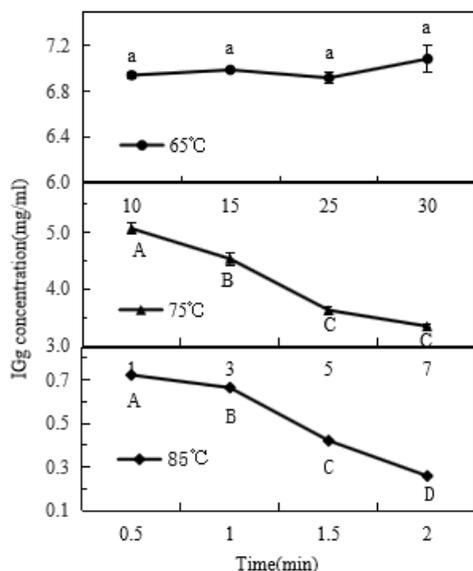


Figure 5. Changes of IgG with heating temperature

But when the goat colostrum was heated at 75°C for 5 min, there was a significant decrease in IgG concentration ($p < 0.01$). Heat treatment at a temperature higher than 72°C caused a loss in immune activity and a change in the secondary structure of bovine IgG at neutral pH [10]. The IgG was almost completely inactivated after heated at 85°C for 2 min. Ustunol et al. had found that IgG was the most stable immunoglobulin at 70°C and that there was a sharp decrease in the activity of IgG within the first 5 min at 85°C [11]. These results suggest that pasteurization processes do not necessarily result in complete destruction of IgG, and IgG in colostrum has good thermal stability at 65°C.

3.5 Analysis on the effect of pH on IgG

The effect of pH on the IgG concentration in goat colostrum was displayed in Figure 6. The maximum IgG level was observed at pH 6.5. At pH levels less than 6.5, the concentration of active IgG decreased by approximately 20% of the maximum value at pH 4.0. At pH more than 7.5, in an alkaline environment, IgG levels significantly dropped and were only 60% of the maximum when the pH increased to pH 9.5. No IgG activity was detected for a pH lower than 4.0 or higher than 10.0 [12]. When the pH value is greater than 12, the activity of immunoglobulin G in goose drops rapidly and increases the infection rate of GPV virus [13]. Therefore, IgG had poor stability in both strong acids and strong bases.

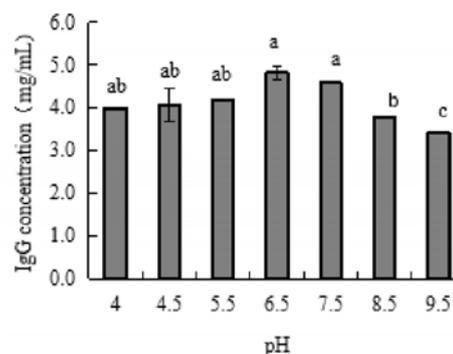


Figure 6. Changes of IgG with pH

3.6 Analysis on the effect of ultra-high pressure treatment on IgG

Figure 7 showed that IgG concentration began to decline rapidly during the first 15 min for all pressure treatment conditions. A remarkably significant reduction was observed as the pressure treatments increased from 200 to 500 MPa at each time point. But, with 500 MPa treatment for 20 min, IgG levels had dropped to about 70% of those treated for 10 min, which were similar to previous report that treatment of 500 MPa caused significant losses of IgG in goat colostrum [14, 15].

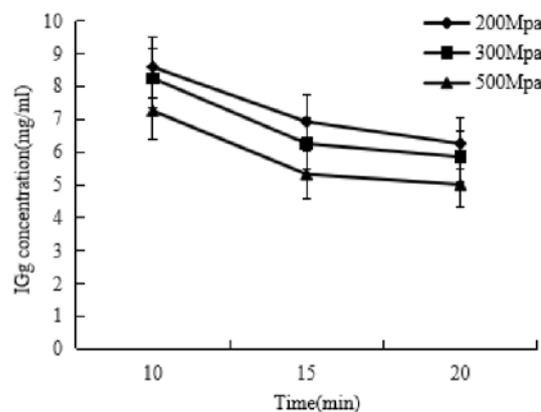


Figure 7. Changes of IgG with ultra-high pressure

3.7 Analysis on the effect of citric acid and calcium salts concentration on IgG

As Figure 8 displayed, with increasing concentrations of citric acid, the concentration of IgG reduced dramatically ($p < 0.05$). The highest concentration of citric acid significantly reduced the pH value of milk samples to about pH 3.5 and these results are consistent with the previous studies [16] demonstrating that the stability of IgG in colostrum is greatly reduced under strong acidic conditions.

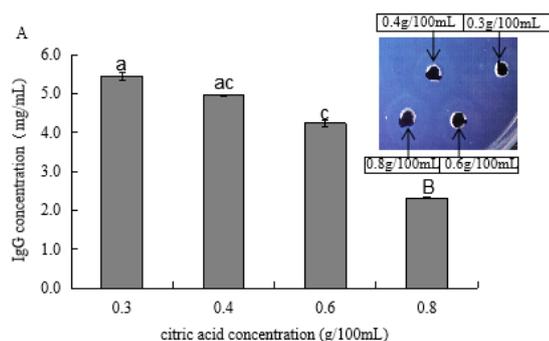


Figure 8. Changes of IgG with the concentration of citric acid

With the concentration of calcium ions increasing, IgG concentration was found to decrease significantly (Figure 9). No IgG was measurable when the concentration of calcium ions was higher than 1.0 g/ml. In general, the more calcium that was added to the colostrum, the worse the stability of the goat colostrum.

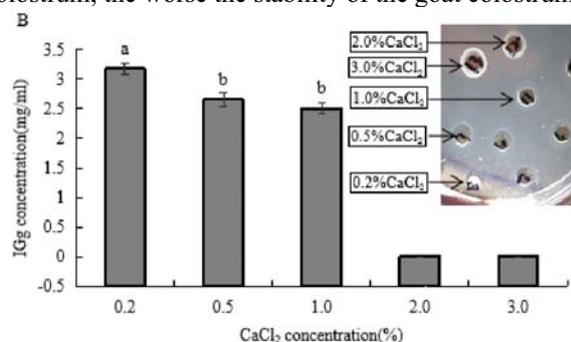


Figure 9. Changes of IgG with the concentration of Ca²⁺

4 Conclusion

In conclusion, the freezing of goat colostrum was a good method of IgG preservation for the first 45 days, and room temperature was of the preferred thawing method for the freezing goat milk. At pH 6.5, the maximum of IgG concentration in goat milk was observed. The IgG concentrations decreased significantly with prolonged lactation, and increasing of the temperature, citric acid, calcium salts concentration, and ultra-high pressure. In this paper, we determine the optimal conditions to maintain maximum immunoglobulin activity for goat milk, and the key parameters controlling the stability of immunoglobulin with high activity can be used in practice for goat milk products processing.

Acknowledgments

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