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Research Article

PURIFICATION AND STANDARDIZATION OF IMMUNOGLOBULIN FROM BOVINE COLOSTRUMS

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ABSTRACT

Colotral antibodies provide passive immunity to the new-born calf and growth factors control some fundamental life process such as cell division, cell proliferation or apoptosis and stimulate the growth and development of the gastrointestinal tract of new –born animals.immunoglobulin particularly IgA , are important for mucosal resistant against bacteria and intestinal parasite. The major Ig present in ruminant milk is IgG, with IgG 1 representing more than 90% , IgA and IgM are found. Our research aim to purify the bioactive protein from bovine colostrums by fractionation using ammonium sulphate yielded 270 µg/ 100µl of globulin. When double purification was used for the fractionation the globulin yield in case of bovine colostrums was found to be 270 µg/ 200 µl. The fractionation with CA, the yield of globulin in case of bovine colostrums was found to be maximum 140 µg/ 500 µl. In SDS PAGE analysis, all samples were processed and shown clear band 97 KDa.

INTRODUCTION

Bovine colostrum is the pre-milk fluid produced by cow mammary glands during the first two to four days after giving birth. Bovine colostrums deliver growth, nutrient, and immune factors to the offspring [1]. It contain many essential nutrient and bioactive components, including growth factors (essentially insulin like growth factor-1(IGF-1), transformation growth factor(TGF), growth hormone(GH), immunoglobulins (Igs), lactoperoxidase (LP), lysozyme (Lys), lactoferrin(Lf), cytokine, nucleoside, vitamin, piptides, and oligosaccharides, which are of increasing relevance to human sheath [2]. Most research increased on bioactive compounds from natural foods by pharmaceutical and functional food industries to meet the demands people are interested in health prevention [4, 5].

Traditional uses of bovine colostrums include for eye conditions, oral health, and respiratory tract infections. The investigation of clinical effects of bovine colostrums in humans began in the late 1980s and continues today [6,7, 8]. Bovine colostrums may be useful for exercise performance enhancement and gastrointestinal injury due to bowel disease and non-steroidal anti-inflammatory drugs (NSAIDs). Hyper immune bovine colostrums is also commercially available, and some evidence exists for its use, as well as the use of isolated immunoglobulin's (antibodies). Most evidence is in support of its use for diarrhea associated with certain types of bacterial and viral infections or immune system deficiencies [3,9].

Colostral whey protein concentrate (70% proteins, most >10,000 Daltons molecular weight) is considered similar to whey protein concentrate, a direct food substance affirmed as generally recognized as safe (GRAS) as described in 21 CFR 184[10, 12, 13]. This "early" milk has a nutrient profile and immunological composition substantially different from "mature" milk. In addition to the macronutrients found in milk (protein, carbohydrate, fat, vitamins, and minerals), colostrums contains oligosaccharides, growth factors, antimicrobial compounds, and immune-regulating constituents either not present in milk or present in mature milk in substantially lower concentration [11,14].

Human milk in place of serum in cell culture supports normal growth of various cell types, such as epithelial cells, fibroblasts and smooth muscle cell. The concentration of immune factors is all so much higher in colostrums than in milk [15, 18]. Colostrums helps to supports health immunity, marrow, neuron, digestion and muscle function. Colostrums is useful for treating a wide variety of intestinal disorders, including inflammatory bowel diseases, nonsteroidel anti inflammatory drug, (NSAIB) induced gut injury, viral gastroenteritis and chemotherapy induced mucositis. Several clinical studies have suggested that bovine colostrums may have anti-inflammatory effect in various intestinal inflammatory disorders [16, 17].

Separation and purification of colostrums protein due to their wide application in food industry, medical and as supplements, large scale production system for the downstream processing of

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recombinant antibodies. Immunoglobulin- rich fractions are usually prepared by removing fat and casein followed by centrifugation process [19]. Sometime lyophilization or spray drying method. to further utilize bioactive substances such as bovine colostrums IgA and IgG , a procedure including salting out (saturated ammonium sulphate method) ,chemical method (caprylic acid precipitation method) ,centrifugation method on isolation purification of bovine colostrums [20]. The industrial production depending on the market requirement, other procedures may be employed as the suitable steps for the products commerciality, such as freeze drying and crystallization [21,22]. Therefore, the protocols for the purification of proteins should be designed according to the feed stock and final requirement. Although a wide variety of protocols can be used to separate bioactive protein from complex food stock, single radial immune diffusion method, SDS-PAGE electrophoresis, chromatography method, some other immune electrophoresis can be use.

MATERIAL AND METHODS

Collection of colostrums

Bovine colostrums sample were collected with the first day after cow parturition and sample were immediately frozen and stored at- 4^{o}C.

Preparation of whey

The frozen samples were thawed and liquid fractions were removed by centrifugation at 2000 rpm for 30 minutes. Acid colostrums whey prepared by precipitation of the casein from skimmed colostrums with 1mol / L HCL at pH 4.2. The casein was removed by centrifugation at 4000 rpm for 10 minutes. The whey was collected.

Purification of immunoglobulin from bovine colostrums

In this research study, the purification of bovine colostrums was purified chemically using ammonium sulphate (AR Himedia) and Caprylic acid (GR Himedia). Ammonium sulphate and Caprylic acid was also under taken for quality and quantity comparison and efficacy of purification processes.

Purification of immunoglobulin by ammonium sulfate method

Saturated Ammonium sulfate (GR Himedia) solution of pH 7.0 was added to the diluted colostrums whey slowly with constant stirring. This was the first precipitation step. In this step, unwanted protein fractions were precipitated out. The mixture was kept overnight to complete precipitation. Next day, the precipitated colostrums was filtered through chain cloth bags and the precipitates were washed again with Ammonium sulfate solution. The dialyzed serum, 0.85% (w/v) NaCl was added to make it isotonic condition. The pH of the purified immunoglobulin preparations was adjusted to 7.0 with 10% (w/v) NaoH and stored at 2-8^{o}C. The purified serum was estimated for total protein, albumin and globulin [25].

Purification of immunoglobulin by caprylic acid method

5 ml of bovine colostrum were diluted with distilled water. The pH of the reaction mixture was brought to 5.0 by adding 10 % NaOH (v/v). The caprylic acid was added very carefully and slowly with constant stirring and the resulting reaction mixture was then stirred vigorously for 60 minutes at 22-24^{0} C. The mixture was filtered through chain cloth to remove the precipitate and the filtrate was then dialyzed by using cellophane bags to remove completely n-Octanoic acid [25].

Purification of immunoglobulin by Ammonium sulfate and Caprylic acid method

Saturated Ammonium sulfate (GR Himedia) solution of pH 7.0 was added to the diluted colostrum whey slowly with constant stirring. This was the first precipitation step. In this step, fibrinogen and most of the euglobulin fraction were precipitated out. The mixture was kept overnight to complete precipitation. Next day, the precipitated plasma was filtered through chain cloth bags and the precipitates were washed again with Ammonium sulfate solution. The pH of the reaction mixture was brought to 5.0 by adding 10 % NaOH (v/v). The Caprylic acid (GR, Himedia) was added very carefully and slowly with constant stirring and the resulting reaction mixture was then stirred vigorously for 60 minutes in room temperature. The mixtures were then filtered through chain cloth bag and the filtrates were dialyzed by using cellophane bag to remove Caprylic acid. Finally total protein estimation and purity analysis were done by Lowry's method and SDS-PAGE respectively.

RESULTS AND DISCUSSION

In this present research study is immunoglobulin purification carried out in bovine colostrums. The quality of plasma purified by ammonium sulphate and Caprylic acid was carried out. The idea of purification was to remove the maximum quantity of unwanted product and to retain maximum quantity of globulin.

Ammonium sulphate fractionation method

The total protein, albumin, globulin, yield, velocity of filtration and turbidity of the rabies antiserums are given in table-1. The total protein yield, velocity of filtration and turbidity of the colostrums immunoglobulins are given in table-1. In case of bovine colostrums containing immunoglobulin yield, the final protein content was found to be more (270µg/100µl). The casein content needs to be optimally removed while retaining the maximum quantity of globulin[5].

Caprylic acid purification method

The new chemical method using caprylic acid was carried out either with or without pepsin digestion. The effect of caprylic acid concentration on the fractionation of human blood plasma obtained is given in table -2. The maximum yield of globulin 140µg/500µl and initial content of protein is 140µg/500 µl. At this concentration, the globulin was not turbid and the flow of filtration was fast [25]. The recovery of globulin yield was found to be more.

Ammonium sulphate and Caprylic acid fractionation

The total protein yield, velocity of filtration and turbidity of the colostrums immunoglobulins are given in table-3. The yield of globulin content 270µg/200µl because it is one of the double purification methods. The velocity of filtration is fast and turbidity is moderate level.

Table 1 Effect of ammonium sulfate fractionation of bovine colostrums

Milk source	Volume of sample (µl)	Absorbence	Final conc.protein (µg)	Velocity of filtration	Turbidity
Cow	100	0.4319	270	Fast	++

**Fast: less than 10 minutes; slow- between 10-20 minutes*
(++)- moderate turbidity; (-)- no turbidity

Table 2 Caprylic acid purification of bovine colostrums

Milk source	Volume of sample (µl)	Absorbence	Final conc.protein (µg)	Velocity of filtration	Turbidity
Cow	500	0.2234	140	Fast	++

**Fast: less than 10 minutes; slow- between 10-20 minutes*
(++)- moderate turbidity; (-)- no turbidity

Table 3 Effect of ammonium sulfate and caprylic acid fractionation of bovine colostrums.

Milk source	Volume of sample (µl)	Absorbence	Final conc.protein (µg)	Velocity of filtration	Turbidity
Cow	200	0.6370	270	Fast	++

**Fast: less than 10 minutes; slow- between 10-20 minutes*
(++)- moderate turbidity; (-)- no turbidity

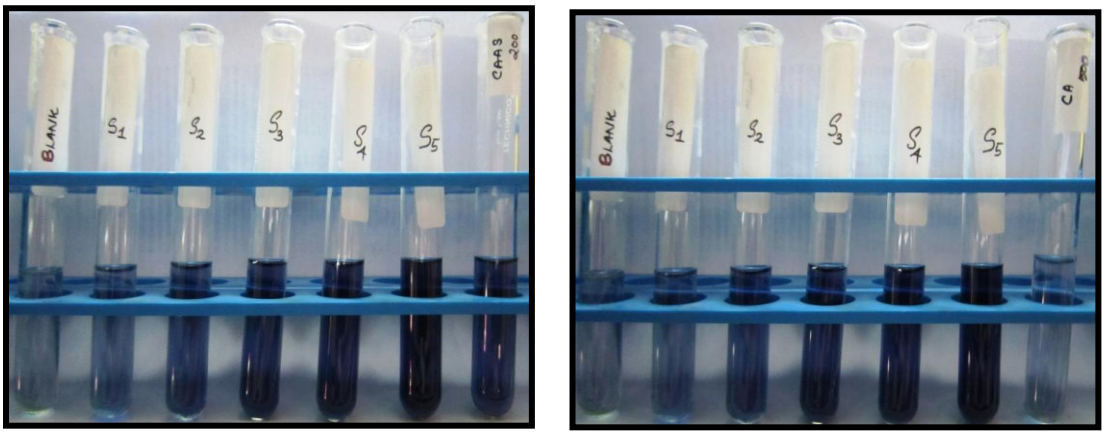

Fig.1. Protein estimation of Ammonium sulphate(A) and Caprylic acid (B) purified samples

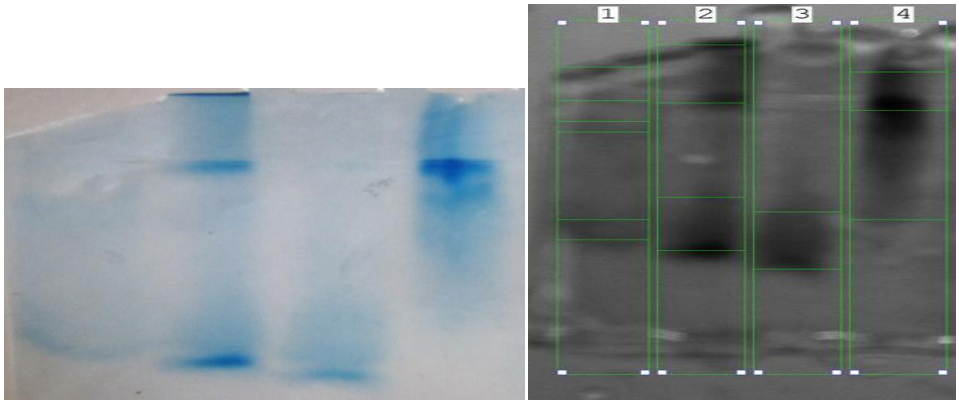

Fig.3 Purity analysis by SDS PAGE

Purity analysis by SDS- PAGE

After the purification of bovine colostrums immunoglobulin purity has been analyzed by SDS PAGE. Each samples shown better result and all samples contain the IgG immunoglobulion protein bands were observed between 90 -110 KDa (Fig.3).

IgG one of the major immunoglobulin, that mainly used for the improve the nutritional value and it may help to provide immunity to infants etc [22].

CONCLUSION

Pasteurization of colostrums could serve as an effective and practical method of reducing pathogen exposure to highly susceptible newborn calves, reducing the high rate of preweaning mortality (8.4 to 10.7%) experienced in dairy replacement heifers in the United States (National Animal Health Monitoring System, 1993, 1996, 2002) as well as reducing the risk for transmitting specific economically important pathogens. Present investigation was developed new techniques for the Purification of bioactive protein from bovine colostrums by fractionation using ammonium sulphate yielded 270 µg/ 100µl of globulin. When double purification was used for the fractionation the globulin yield in case of bovine colostrums was found to be 270 µg/ 200 µl. The fractionation with CA, the yield of globulin in case of bovine colostrums was found to be maximum 140 µg/ 500 µl. In SDS PAGE analysis, all samples were processed and shown clear band 97 KDa. This picture also indicates IgG immunoglobulin molecular range. Lane1, lane 2 and lane 3has shown brisk band in 90-94 KDa.In future, we have planned to develop commercial products from colostrums.

References

1. Arguello, A., Castro, N., Batista, M., Moreno-Indian, I., Moreles-Delonuez, A., Sanchez Macias, D., Quesada, E., & Capote, J. (2008). Chitotriosidase activity in goat blood and colostrums. *Journal of Dairy Science, 91*(6), 2067-2070.
2. Arand, P., Sanchez, L., Perez, M., Ena, J. M., & Calvo, M. (1991). Insulin in bovine colostrums and milk: Evolution throughout lactation and binding to caseins. *Journal of Dairy Science, 74*(11), 4320-4325.
3. Brussow, H., Hilper, H., Walther, I., Sidoti, J., Mietens, C., & Bachmann, P. (1987). Bovine milk immunoglobulin for passive immunity to infantile rotavirus gastroenteritis. *Journal of Clinical Microbiology, 25*(6), 982-986.
4. Butles, J. E. (1983). Bovine immunoglobulin: A augmented review. *Veterinary Immunology and Immunopathology, 4*(1), 43-52.
5. Cairangzhuoma, Yamamoto, M., Muranishi, H., Inogaki, M., Uchida, K., Yamashita, K., Saito, S., Yabe, T., & Kanamaru, Y. (2013). Skimmed, sterilized, and concentrated bovine late colostrum promotes both prevention and recovery from intestinal tissue damage in mice. *American Dairy Science Association*, 1347-1355.
6. Donahue, M., Godden, S. M., Bey, R., Wells, S., Oakes, J. M., Sreevatsan, S., Stabel, J., & Fetrow, J. (2012). Heat treatment of colostrums on commercial dairy forms decreases colostrums microbial counts while maintaining colostrums immunoglobulin G concentrations. *Journal of Dairy Science, 95*(5), 2697-2702.
7. Francis, L., Read, C., Ballard, F., Bagley, J., Upton, M., Gravestock, M., & Wallace, C. (1986). Purification and partial sequence analysis of insulin-like growth factor from bovine colostrums. *Biochemical Journal, 233*(1), 207-213.
8. Graczyk, T. K., Cranfield, M. R., Helmer, P., Fayer, R., & Bostwick, E. F. (1998). Therapeutic efficacy of hyperimmune bovine colostrums treatment against clinical and sub-clinical cryptosporidium serpentis infection in captive snakes. *Veterinary Parasitology, 74*(2-4), 123-132.
9. Godden, S., McMartin, S., Feirtag, J., Stabel, J., Bey, R., Goyal, S., Metzger, L., Fetrow, J., Wells, S., & Chester-jones, H. (2006). Heat treatment of bovine colostrums: Effect of heating duration of pathogen viability and immunoglobulin G. *American Dairy Science Association, 89*(9), 3476-3483.
10. Health, W., Neave, L. V., & Veira, D. M. (2013). Preparation of a high immunoglobulin product from bovine colostrums. *Animal Feed Science and Technology, 197*, 133-137.
11. Huang, J., Yan, Q., & Yan, Z. (2001). Ion exchange and protein G affinity column in tandem for the separation of protein. *Journal of Biochemical and Biophysical Methods, 49*(1-3), 263-273).
12. Jeongrai Lee, Hyun-Mi Kim, Hsseun Choi, & Jeong Hwa Hong. (2007). Effect of colostrums basic protein from colostrums whey protein: Increased in osteoblast proliferation and bone metabolism. *Journal of Food Science and Nutrition, 12*(1), 1-6.
13. Korhonen, H. (1998). Colostrum immunoglobulins and the complement system – potential ingredients of functional foods. *Bulletin International Dairy Federation, 336*, 36-40.
14. Kathleen, F., Benson, S. G., Carter, S. G., Patterson, K. M., Patel, D., & Jensen, G. S. (2012). A novel extract from bovine colostrums whey supports anti-bacterial and anti-viral innate immune function in vitro and in vivo. 1. Enhanced immune activity in vitro translates to improved microbial clearance in animal infection models. *Preventive Medicine, 54*, 5116-5123.
15. Katsuro Hagiwara, Satoshi Kataoka, Hitoki Yamanaka, Rikio Kirisawa, & Hiroshi Iwai. (2000). Detection of cytokines in bovine colostrum. *Veterinary Immunology and Immunopathology, 76*(3-4), 183-190.
16. Keho, S. I., Jayarao, B. M., & Heinrichs, A. J. (2007). A survey of bovine colostrums composition and colostrums management practices on Pennsylvania dairy farms. *Journal of Dairy Science, 90*(9), 4108-4116.
17. Loste, A., Ramos, J. J., Fernandez, A., Ferrer, L. M., Lacasta, D., Verde, M. T., Marca, M. C., & Ortin, A. (2008). Effects of colostrum treated by heat on immunological parameters in newborn lambs. *Livestock Science, 117*(2-3), 176-183.
18. Lidia Elfstrand, Helena Lindmark-Mansson, Marie Paulsson, Lena Nyberg, & Bjorn Akesson. (2002). Immunoglobulin, growth factor, and growth hormone in bovine colostrums and the effects of processed. *International Dairy Journal, 12*(10), 879-889.
19. Mbuthia, E. W., Klobasa, F., Gachuiri, C. K., & Abate, A. (1997). Effects of treatment with formaldehyde and formic on immunoglobulin content of stored bovine colostrum. *Animal Feed Science and Technology, 67*(3), 291-298.

20. Mohanty, J. G., & Elazhary, Y. (1989). Purification of IgG from serum with caprylic acid and ammonium sulphate precipitation is not superior to ammonium sulfate precipitation alone. *Comparative Immunology, Microbiology and Infectious Diseases, 12*(3), 153-160
21. Morrill, K. M., Conrad, E., Lago, A., Campbell, J., Quigley, J., & Tyler, H. (2012). Nationwide evolution of quality and composition of colostrums on dairy forms in the United States. *Journal of Dairy Science, 95*(7), 3997-4005.
22. Jayabalan Jayaprakash. (2013). Production of rabies immunoglobulin using caprylic acid purification. *St. Joseph's Journal of Humanities and Science, 1*(1), 98-102.
23. Pakkanen, R., & Aalto, J. (1997). Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal, 7*(4-5), 285-297.
24. Quigley, J. D., Lago, A., Chapman, C., Erickson, P., & Polo, J. (2013). Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. *Journal of Dairy Science, 96*(2), 1148-1155.
25. Rojas, G., Jimenez, J. M., & Gutierez, J. M. (1994). Caprylic acid fractionation of hyperimmune horse plasma: Description of a simple procedure for antivenom production. *Toxicon, 32*(3), 351-363.

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