Int. J. Drug Dev. & Res.
| October - December 2013 | Vol. 5 | Issue 4 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Strategic Studies on Colostrum (Junnu) Collected from Katpadi , Southern Tamil Nadu for Pharmacological Use

Chacko Anu Betty*

Lina Rose Varghese

Jennifer T Thomas

Suneetha V

School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India

Corresponding Authors:
Ms Chacko Anu Betty
C/o. Dr Suneetha V
Associate Professor and Youth Red Cross coordinator
Room no-108,115, Hexagon
VIT University, Vellore, Tamilnadu, India -632014
email- vsuneetha@vit.ac.in
Web: http://www.vit.ac.in/home.asp

Abstract:
Colostrum milk is an all-round liquid food that contains biologically active compounds other than carbohydrates, proteins and fatty acids like vitamins, immunoglobulin, enzymes, growth hormones, and minerals that form an essential component of our biological system. Colostrum milk is the first milk of the cow's udder that seems to provide all these essential nutrients. The extraction techniques lead to separation of major biological compounds such as fat soluble, water soluble nutritive constituents which gives the idea of synthesizing a product that can be easily consumable to people. Various nutrients that constitute the colostrum milk are extracted from various sources to chemically synthesize a valuable product that could support and nourish human health. Several techniques of purification are carried out using an extraction protocol. High pressure liquid chromatography, SDS PAGE and bioassay techniques were used to identify nutrient of interest. This product is prepared devoid of lactose. Rapid sensitive separation and analysis was achieved combining all these contents to protect intolerance and supplement children from malnutrition. Plant sources that include green gram, cereals, green leafy vegetables and that of animal origin such as egg and colostrum milk are used to chemically synthesize milk. This supports human health, help relieve from possible infections and enable health nourisble.

Keywords: Colostrum, biologically active compounds,immunoglobulin, growth

Introduction

Colostrum milk is the first milk extracted from the cow's udder. This is very nutritious in quality in the sense that it contains not only the basic macromolecules of the diet but contains peptides like lactoferrin, β-lactoglobulin, a-lactalbumin. It also contains nonspecific nutrients namely immunoglobulin like IgG, IgM, and IgA, essential growth hormones like insulin, prolactin, thyroid hormones, cortisol, prostaglandins, enzymes, cytokines(tumor necrosis factor), nucleotides, polyamines, minerals like iron, magnesium, sodium salts, water and fat soluble vitamins as well as epithelial cells, lymphocytes and monocytes. Milk contains two primary sources of protein, the caseins and whey. After processing occurs, the caseins are the proteins responsible for making curds, while whey remains in an aqueous environment [1,21].The components of whey include β-lactoglobulin, a-lactalbumin, bovine serum albumin, lactoferrin, immune globulins, lactoperoxidase enzymes, glycomacropeptides, lactose, and minerals [2,18,20]. In additional, whey derived from buttermilk versus cheese contains the lipid sphingomyelin. Historically, whey was considered a cure-all used to heal ailments ranging from gastrointestinal complaints to joint and ligament problems. Today, whey is a popular dietary protein supplement purported to provide antimicrobial activity, immune modulation, improved muscle strength and body composition, and to prevent cardiovascular disease and osteoporosis. Advances in processing technology, including ultra-filtration, microfiltration, reverse

osmosis, and ion-exchange, have resulted in development of several different finished whey products. Whey protein concentrates (ranging from 80-95 percent protein), reduced lactose whey, whey protein isolate, demineralized whey, and hydrolyzed whey are now available commercially.

Lactoferrin, an iron-binding glycoprotein, is a non-enzymatic antioxidant found in the whey fraction of milk as well as in colostrum. Colostrum contain a greater concentration of immunoglobulins. Similarly, the whey fraction of milk appears to contain a significant amount of immune globulins. The authors further conclude bovine milk IgG typically ranges between 0.6-0.9 mg/mL and is therefore likely to confer immunity that could be carried to humans. Studies show raw milk from non-immunized cows contain specific antibodies to human rotavirus, as well as antibodies to bacteria such as *E. coli*, *Salmonella Enteriditis*, *S.typhimurium*, and *Shigellaflexneri*[3,4]. Studies on lactoferrin have demonstrated its ability to activate natural killer (NK) cells and neutrophils, induce colony-stimulating factor activity, and enhance macrophage cytotoxicity. Lactoferrin also appears to have antiviral, antifungal, and antibacterial properties. The antimicrobial effect is likely more potent in organisms that require iron to replicate, as Lactoferrin has the unique ability to chelate iron in a way that deprives microorganisms of this essential nutrient for growth [5,19]. In additional, Lactoferrin has the ability to release the outer membrane of Gram negative bacteria, the lipopolysaccharide component, thus acting as an antibiotic [6]. Lactoferrin demonstrates anti-inflammatory properties.

β-Lactoglobulin represents approximately half of the total protein in bovine whey being a source of essential and branched chain amino acids, a retinol-binding protein has been identified within the β-lactoglobulin structure. α-Lactalbumin has enhanced antibody response to systematic antigen stimulation. It has a direct effect on B-lymphocyte function, as well as suppressing T cell-dependent and –independent responses. Lactoperoxidase has the ability to catalyze certain molecules, including the reduction of hydrogen peroxide [7]. This enzyme system catalyzes per oxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species. During the pasteurization process, lactoperoxidase is not inactivated, suggesting its stability as a preservative.

MATERIALS AND METHODS

Standard Solutions

Water soluble vitamins were prepared at 1 mg/ml (concentrations B1 -1mg, B2- 1mg, B6-0.5mg, B12-1mcg, nicotinamide -15mg) in 10mM ammonium acetate (pH 4.5) and kept at 4°C until further analysis. Fat soluble vitamins were prepared at 0.1 mg/ml with ethyl acetate. All chemicals and reagents were stored at refrigerator and freshly taken at the time of analysis. Immunoglobins and protein standard samples were all stored at 4°C.All the chemicals used for the chromatographic techniques were of HPLC grade. Analysis by PAGE used were prepared freshly and used at the time prior to running the gel. Care should be taken that these must not be exposed to extreme light or temperature conditions.

Colostrum milk sample was defatted by centrifugation at 15000rpm at 4°C, 30 minutes. The fat layer was removed and gently homogenized at 50°C. The samples were shaken for 1minute and

ultra sonicated for 5 minutes. Acetic acid was used to adjust the pH of the extracts to 4.5 and then centrifuged for 5 minutes at 4000rpm. The upper layer was adjusted to pH 7 with NaOH (1M) and filtered through 0.45µm syringe filter. This filtrate was analyzed using HPLC.

Green gram seeds were washed and soaked in distilled water overnight at room temperature for germination. Then the seeds were ground by electric homogenizer using cold acetone to remove the fat and filtered by suction through double ring filter papers. Then homogenates were air dried overnight. After that, seeds were powdered finally under ice cold condition using mortar and pestle and finally suspended in 10mMTrisHCl buffer at pH 8 containing 2M NaCl for 4 hours. The extracted mixture was filtered using gauge. Filtrate was centrifuged at 10000 rpm for 8 minutes at 4°C. The supernatant was analyzed. Cereal samples consisting of rice flour, soya flour and corn flour were ground and homogenized. 1 gram was weighed into 50ml beakers. 2ml of 0.1M HCl was added to the beaker and was heated in a water bath at 100°C for 20 minutes. This treatment solubilizes the contents. Cool the contents to ambient temperature. Adjust the volume to 1litre with de ionized water. Filter the contents through a 0.45µm filter membrane. The sample was also prepared by treatment with enzymes such as diastase or papain to release the vitamins from their conjugated forms and was adjusted to 10ml. At high concentrations there was interference of salts, as a result the sample were diluted further and analyzed by HPLC.

Eggs were also used for analysis. Egg yolk was separated from egg white. 1ml of egg yolk was diluted to 10 ml with distilled water. The solution was mixed and stirred for 6hrs, 4°C. The solution was centrifuged at 8,000 rpm 4°C for 25 min. Supernatant was collected and potassium sulphate solution was added with constant stirring. The pH was adjusted to 7.5 and the sample was filtered using a 0.45µm filter. Analysis was done by SDS PAGE [8,9].

Fat soluble vitamins such as vitamin A were extracted simultaneously with vitamin E from green leafy vegetables such as lettuce, cabbage [10, 11,13,15]. About 0.50 grams of the finely ground leaves was mixed 16ml of 10mM ammonium acetate/methanol 50:50(v/v) containing 0.1% butylated hydroxyl toluene (BHT). Solutions of hippuric acid and trans-ß-Apo-8'-carotenal was added (5µg/ml and 3.3µg/ml). Sample kept at ice condition (15minutes) and centrifuged at 8000rpm for 10minutes. Supernatant was preserved. This was passed through nitrogen stream to evaporate methanol. Residue was extracted once again with ethyl acetate and 0.1% BHT (6ml+6ml) for 15 minutes. Samples were shaken for 15minutes and were placed in water bath to ensure the temperature does not exceed above 25°C. The two supernatants were centrifuged at 8,000rpm for 15 minutes and the supernatant was filtered through 0.45µm filter. Extract was placed in nitrogen stream to dryness and the residue was further dissolved in ethyl acetate and injected into HPLC [12,15,16,17,19].

PAGE was performed in 3-27% poly acrylamide gel containing 1% SDS. Aliquots of 20µl was mixed with 75µl sample buffer (1M TrisHCl/L,0.1% SDS pH 6.8,0.25% bromophenol blue, 2.5% β-mercapto ethanol was added, mixed and heated in boiling water for 3 mins.15µl of the sample were loaded on the gel. Slab gels were run in 1M Tris/glycine buffer/l (pH 8.3) with 0.1% SDS at 35mA constant current for about 4 hrs. Once the dye has reached 3/4th front of the gel, running is discontinued. Gels

were then further stained overnight in 300ml solution of acetic acid: ethanol: water containing 0.35g coomassie brilliant blue. The de staining in ethanol: acetic acid: water was then used to obtain a clear background.

Chromatography

HPLC: Isocratic

Mobile phase A: 75:0.5 (water: glacial acetic acid)

Mobile phase B: Methanol

Total flow rate 1ml/min (70% A: 30% B)

Absorbance at 282nm

Pressure: 40 MPa (Option 2)

Gradient

A-97%: B-3%

Mobile phase A: 10mM ammonium formate (pH 3.47)

Mobile phase B: Methanol

Temperature at 40°C

Sample temperature: 4°C

Injection volume: 1µl

Pressure: 40MPa

Assay Techniques

Vitamin A levels have been determined using colorimetric and spectrophotometric methods for a long time. Currently, HPLC is the method of choice. The colorimetric method involves adding a chromogenic reagent to a volume of solubilized fortified food sample. The reagent reacts with retinol to produce a blue color, whose intensity is proportional to the amount of retinol in the sample. The intensity of the blue color is measured against a set of known standards.

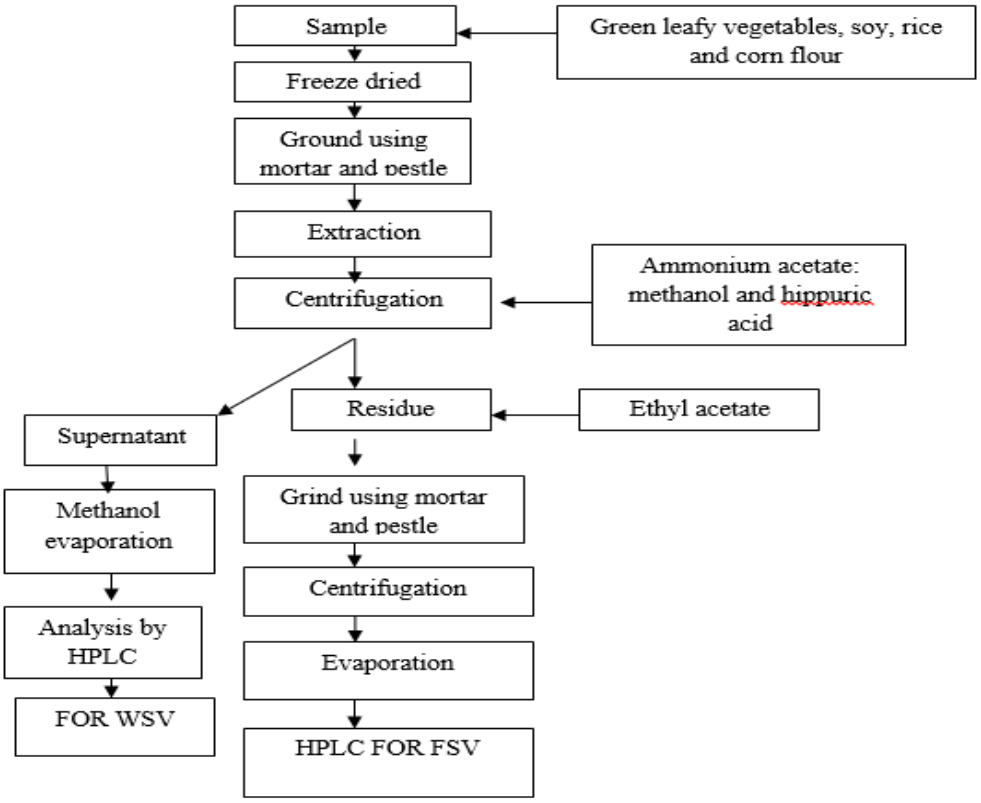
The sample is irradiated with UV light and its absorbance is measured. The absorbance is proportional to the vitamin A content in the sample. The spectrophotometric method can be used to monitor vitamin A levels in fortified products at the production level.

Thiamin (vitamin B1) is analyzed quantitatively by fluorometric methods. The method of choice is the thiochrome procedure, which involves treatment of thiamin with an oxidizing agent (ferricyanide or hydrogen peroxide) to form a fluorescent compound (thiochrome). The intensity of fluorescence is proportional to the thiamin concentration.Riboflavin (vitamin B2) is usually assayed flurometrically by measuring its characteristic yellowish green fluorescence.Niacin is assayed semi-quantitatively with sulfanilic acid to yield a yellow color. The intensity of the yellow color correlates with the amount of niacin present, which is measured against a set of standards.High pressure liquid chromatography (HPLC) methods to determine most B-complex vitamins have been considered and evaluated. Vitamin E levels can be determined spectrophotometrically, although the HPLC method with fluorescence detection is preferred.

Table 1: Comparison of Techniques

Techniques	Advantages	Disadvantages
Colorimetric	Simple Rapid Inexpensive	Treatment of sample
Spectrophotometric	Inexpensive Sensitive Rapid	Needs UV Apparatus
HPLC	High resolution Time consuming Highly sensitive	Expensive Treatment of sample Requires expert personnel

Chart 1: Extraction protocol for WSV and FSV



Results

The standard samples for water and fat soluble vitamins and immunoglobins were prepared in methanol or 10mM ammonium acetate. All the chemicals were freshly prepared and stored at 4°C. The samples prepared from colostrum milk were successfully extracted. The homogenization separated the fatty layer from milk and pH was made to 4.5 to precipitate out the casein fraction from milk. The whey obtained on centrifugation filtered through 0.45µm membrane filter was stored at ice cold conditions. A control was loaded in the first lane and the remaining lanes containing samples of whey extracted from colostrum milk. From each of the lanes, bands were visible on staining overnight and de staining with a clear background. The gel led to separation of the major whey proteins, albumin, α-lactalbumin, β-lactoglobulin, lysosyme and lactoferrin in the corresponding wells and visualization of control marker in the first lane. According to the literature work, since albumin has a molecular weight of 66kDa, the followings samples that were separated on the gel shows dense bands in the range 27 to 40kDa with a single band that lies on top of the column. This weighs highest and corresponds to lactoferrin. The chromatographic technique demonstrated the high reproducibility of HPLC. The graphs notably showed the differences in the heights of various peaks. The two techniques compared showed similarities in results.

A similar polyacrylamide separation was performed with green gram seeds. The extract was prepared by treatment with acetone. The contents were dissolved in TrisHCl containing NaOH and were further filtered to obtain the filtrate. Separation of proteins of containing a higher number of amino acid residues was observed on electrophoresis. On chromatographic separation, the peaks were

obtained. In comparison with standard vitamin samples the peaks were compared, this demonstrated the presence of water soluble vitamins and proteins. The method gave highly reproducible results that appeared in a very rapid and with low estimation time

The cereals of rice flour, soya flour and corn flour analyzed for HPLC gave reliable results indicating the presence of water soluble vitamins. Thiamine, pantothenate, pyridoxine, nicotinamide, cyanocobalamine, folic acid and riboflavin were detected in cereals.

The egg yolk samples also gave reproducible results which were found to be very reliable. It was very interesting to note that separation of egg yolk sample were reproducible. It was found that egg yolk could be used to extract high amount of nutrients for the preparation of highly nutritious milk. It also gave viable information of containing immunoglobinsIgY. Yolk samples gave high number of bands on electrophoresis and SDS PAGE analysis gave a high purity of 70%.

The extraction of fat soluble vitamins from green leafy samples made it possible for us in isolation of another constituent for the synthesis of artificial milk. After a possible extraction procedure, samples were made easy to detectable quantities by dissolving in ethyl acetate. Later the samples were detectable by HPLC technique. The possible vitamins isolated were vitamin A and E. The results of the entire assay technique concluded the presence of nutritional constituents in the preparation of artificially synthesized milk.

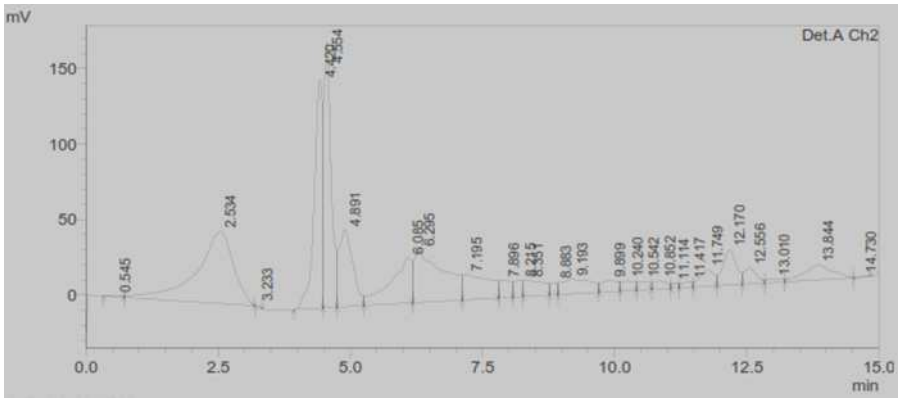


Figure 1: Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B6, Vitamin B12

Table 2: Peak table for standard Vitamin B

Peak#	Ret. Time	Area	Height	Area%	Height %
1	0.546	2838	225	0.089	0.076
2	4.419	1284917	118837	40.447	40.291
3	4.552	1275779	138515	40.160	46.962
4	4.891	613223	37372	19.303	12.671
Total	14.408	3176757	294949	100.000	100.000

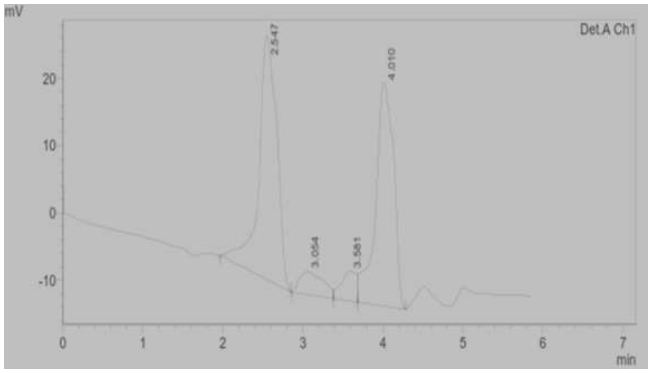


Figure 4: VitaminB3, Vitamin B5 from green gram

Table 4: Peak table for analysis of vitamins from green gram

Peak#	Ret. Time	Area	Height	Area%	Height %
1	2.547	572247	36243	46.724	46.891
2	3.054	72872	3400	5.950	4.399
3	3.581	58897	4389	4.809	5.678
4	4.010	520731	33260	42.517	43.032
Total	13.192	1224747	77292	100.000	100.000

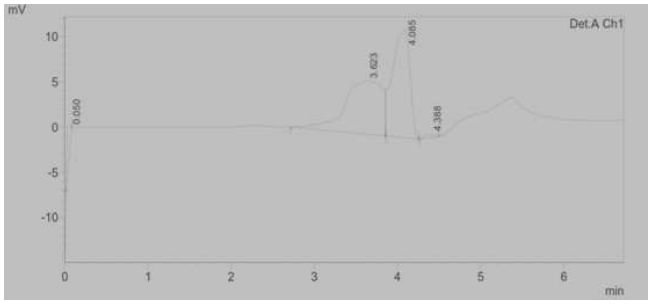


Figure 5: Immunoglobin Y

Table 5: Peak table for the analysis of ImmunoglobinY

Peak#	Ret. Time	Area	Height	Area%	Height %
1	0.050	13604	3404	3.627	15.675
2	3.623	187232	5893	49.915	27.137
3	4.085	171010	11995	45.590	55.237
4	4.388	3257	424	0.868	1.951
Total	12.146	375103	21715	100.000	100.000

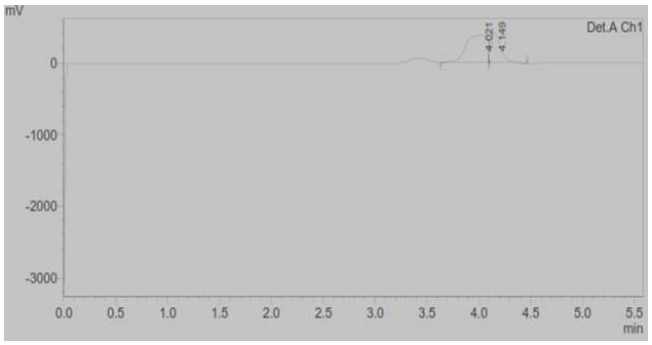


Figure 6: Vitamin E

Table 6: peak table for analysis of vitamin E from green leafy vegetables

Peak#	Ret. Time	Area	Height	Area%	Height %
1	4.021	5482875	383189	66.341	47.915
2	4.149	2781793	416542	33.659	52.085
Total	8.170	8264667	799731	100.000	100.000

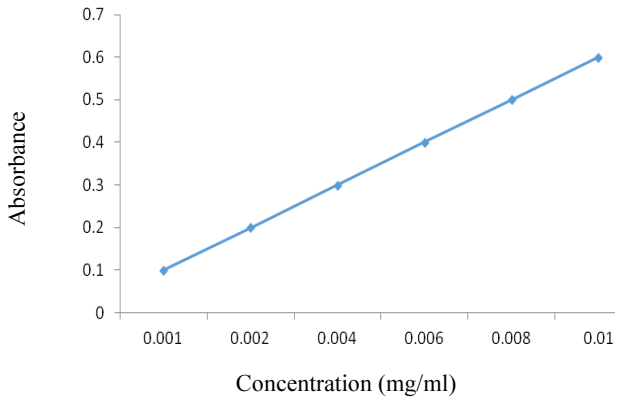
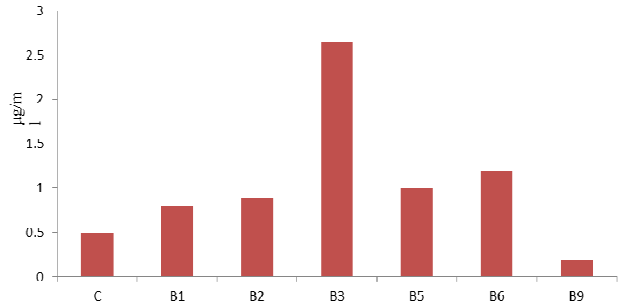


Figure 7: Standard Graph for Vitamins



Mean content of Vitamins B obtained from Soya, Colostrum milk, and green gram.

Figure 8: Mean content of water soluble vitamins

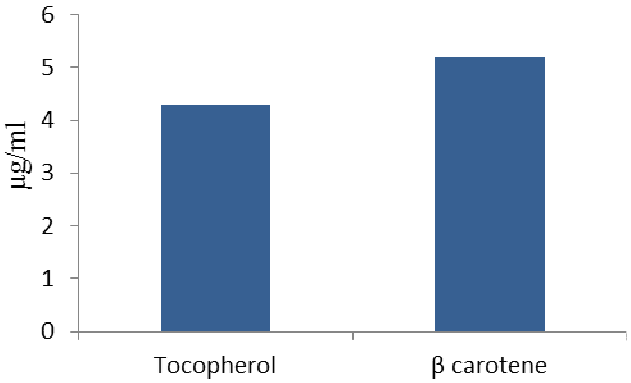


Figure 9: Mean content of vitamin in Egg & green leafy vegetable

Discussion

We have completely focused on colostrum milk as a highly nutritive valuable product for human consumption due to the number of constituents that it possesses. The nutrition present in milk after the delivery of the calf provides all the necessity factors that we could acquire to maintain a healthy life. This milk is regarded as functional

Covered in Scopus & Embase, Elsevier
Int. J. Drug Dev. & Res., October -December 2013, 5 (4): 214-223

food that could have a direct effect on the health of an individual. It contains two parts, one forming the casein proteins that is responsible for curdling and the other forms the aqueous part namely whey. The whey contains β-lactalbumin, lactoglobulin, lactoperoxidase, bovine albumin, immunoglobins, lactoferrin, glycomacropeptides, growth factors and minerals each delivering a specific function. The milk is homogenized to remove the fat and further curdled to separate out the whey fraction. Care was taken to maintain the whey at a cooler temperature so as to maintain the biological activity of whey that contributes to immune enhancing activity. They constitute huge fraction of amino acids that could be easily absorbed and digested. Relative to other proteins, it also constitutes branched chain amino acids and sulphur containing amino acids where the immune function is again enhanced. The immunoglobins present constitutes 10-15% and this supplies the first line of immune defense. β- lactoglobulin has the property to carry hydrophobic molecules such as retinoic acid that modulate lymphatic responses. Alpha lactalbumin contributes affects the B lymphocyte function and suppresses T cell responses. Lactoperoxidase acts as responsible enzyme in reduction of hydrogen peroxide killing a wide range of bacterial species. It's another essential constituent is glycomacropeptides that lacks phenylalanine that is beneficial to individual with phenlketonuria. All together one and other constituent support each other and helps in natural defense throughout the person's life.

The main idea behind this paper was to isolate all the components to possibly synthesize artificial milk that could contribute the same nutritional quality. This offers food product that could be consumed by individual and children lacking essential nutrients and growth factors to essentially maintain long lasting life. During ongoing life there are factors occurring in life or there could be chances that the individual wouldn't have received the essential during their childhood that could cause the deficiency and lead to insufficient levels. This further enabled us to focus on isolation and synthesis to help formulate a product that supports human health.

Conclusion

The milk on the cow's first delivery produces milk that is found to be containing nutritive constituents that can isolated to synthesize a product that is easily consumable to people. There are chances that people might be intolerant to consumption of lactose that is present in milk. To avoid such circumstances, keeping in mind the health benefits of the people, and protect children from malnutrition, product is artificially designed to produce compounds that protect human life from the various infections and hazards of life. Hence isolation was focused from green leafy vegetables, colostrum milk, green gram seeds, cereals and egg.

HPLC was found suitable for the separation and analysis of vitamins, in identification of proteins including immunoglobins. The method required sample pretreatment for HPLC analysis. Polyacrylamide gel electrophoresis gave expected results in separation of proteins with molecular weight ranging from 25-67 kDa. Different spectra photometric and colorimetric methods were performed to assay the presence of vitamins. These parameters were compared with standards obtained from HPLC.

Acknowledgement

The authors wants to express their gratitude to **Dr. G. Viswanathan**, honorable Chancellor, VIT University, Vice Presidents and **Prof Raju V**, Vice chancellor Vellore for their constant support and encouragement and also for providing the infrastructure and good laboratory facilities to carry out this research work and DST India for financial assistance and support.

References

1) Gill HS, Rutherford KJ, Cross ML. Bovine milk: a unique source of immune modulatory ingredient for functional foods. In: Buttriss J, Saltmarsh M, eds. Functional Foods II –Claims and Evidence. Cambridge, England: Royal Society of Chemistry Press 2000; pp.82-90.

2) Walzem RL,Dillard CJ, German JB. Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. Crit Rev Food SciNutr2002; 42:353-375.

3) Losso JN,Dhar J, Kummer A, Li-Chan E, Nakai S. Detection of antibody specificity of raw bovine and human milk to bacterial lipopolysaccharides using PCFIA. Food AgricImmunol 1993; 5:231-239.

4) Yolken RH,Losonsky GA, Vonderfecht S,et al. Antibody to human rotavirus in cow's milk.NEngl J Med 1985; 312:605-610.

5) Shah NP. Effects of milk-derived bioactives: an overview. Br J Nutr 2000; 84:S3-S10.

6) Bjorck L. Antibacterial effect of the lactoperoxidase system on psychotrophic bacteria in milk. Journal of Dairy Research 1978; 45:109-118.

7) R. Vazquez, M-D L. Hoang, J. Martin, Y. A. Yahia, H. Graffard, F. Guyon, B. Do. 536 EJHP Science 2009; 15: 28.

8) Chamow S. and Ashkenazi. Antibody Fusion Proteins, John Wiley and Sons Inc. Publisher, New York 1999.

9) Ed Harlow and Lane D. Using Antibodies: a laboratory manual, Cold Spring Harbor Laboratory Press, New York 1998.

10) P.M. Finglas, U. Faure, P.J. Wagstaffe. Fresenius J. Anal Chem 1993; 345:180.

11) M. Herrero, A. Cifuentes, E. Ibáñez. Comprehensive sampling and sample preparation, J. Pawliszyn Editor, Elsevier, Amsterdam, 2012; p 3.

12) G.M.F. Ball. Vitamins in Foods Analysis, Bioavailability, and Stability, CRC Press, Boca Raton, 2006; p. 3.

13) Mandal Sanjeeb Kumar, Vuppu Suneetha, Preliminary Studies On Probioti Potential Of Selected Lactobacillus VIT SSV Strains Screened From Curd Samples Of Vellore, Bihar, Haryana And Varanasi, International Journal of Pharma and Bio Sciences, 4 (2), 2013,193-200.

14) Suneetha V, Sindhuja K V, Sanjeev K. Screening characterization and optimization of Pullulan producing microorganisms from Chittoor district, Asian Journal of Microbiology Biotechnology and Environmental Sciences, 12(3),2010,149-155.

15) Bishwambhar M, Suneetha V. Characterization of Exopolysaccharide A Pullulan Produced By A Novel Strain Of *AureobasidiumPullulans-Sb-1*Isolated From The Phyllophane of *Brassica Oleracea* Cultivated In Orissa State, India, Asian Journal of Microbiology Biotechnology and Environmental Sciences, 14(3), 2012, 369-374.

16) Sanjay S, Amod K, Suneetha V, Bishwambhar M, Gopinath R, Sharad Y, Bhaskar M, Synthesis and activation of Immobilized beads by natural dye extracts, International Journal of Drug Development & Research 4(1), 2012, 304-310.

17) Suneetha V, Bishwambhar M, Gopinath R, Shrestha S R, Kartik G K B, Pravesh C, Apoorvi C, Kalyani R, Screening and Identification of Degradable Products By Pectin Lyase Producing Actinomycetes from Katpadi And

Chittoor Fruit Industrial Waste Enriched Soil Samples,Asian Journal of Microbiology Biotechnology and Environmental Sciences,14(3), 2012, 405-412.

18) Suneetha V, Raj V. Statistical analysis on optimization of microbial keratinase enzymes screened from Tirupati and Tirumala soil samples, International Journal of Drug Development & Research, 4 (2), 2012,1-6.

19) Suneetha V, Ritika S, Abhishek G, Rahul G. An attempt and brief research study to produce mosquitocidal toxin using *Bacillus Spp.*(VITRARS) isolated from different soil samples (Vellore and Chittor) by degradation of chicken feather wastes.Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3(4), 2012, 40-48.

20) Ram Kishore S, Suneetha V, Screening, Characterisation and *in vitro* Analysis of Scorpion Venom, Asian Journal of Microbiology Biotechnology and Environmental Sciences, 13(2), 2011, 1-4.

21) Rai S, Mehrotra S, Dhingra D, Prasad M, Suneetha V, Preparation of curd in the presence of easily available prebiotic sources and study of their effect on physiochemical, sensory and microbiological properties of the curd, International Journal of Pharmaceutical Sciences Review and Research, 17(1), 2012, 40-43.

Article History: ----------

Date of Submission: 03-04-2013

Date of Acceptance: 29-04-2013

Conflict of Interest: NIL

Source of Support: NONE