

ANALYSIS OF EFFECT OF INDIAN HERBAL AND NATURAL

PRODUCTS ON DAIRY PROTEIN INTOLERANCE

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Abstract:

Different herbal additives like ginger, coriander, coffee, tea were tested for their lactose reduction capacity in different brands of milk and milk products. Ginger and tea extracts were exhibited more lactose reduction activity. The results of this present study reveals that these herbal extracts seems to be involved in the lactose catabolism either directly or indirectly as co-factor for the activity of lactose enzyme, so they may be consumed along with milk products to avoid lactose intolerance. Among the tested milk brands, Amulya was found with higher quantity of lactose (65.7 ppm) and when it's treated with ginger extract it was reduced into 20 ppm.

Keywords : Lactose intolerance, herbal additives.

Intr oduction:

Lactose intolerance is the condition in which the deficiency of lactase essentially needed for the metabolism of lactose. The consequences of this disorder such as bloating, flatulence, stomach cramps and diarrhea. Lactose intolerance of most human races except northern Europeans and some Africans is due to the disappearance of most or all of the lactase activity of the intestinal



persons with lactose intolerance the lactose remaining in the intestinal tract causes discomfort conditions such as diarrhea and formation of intestinal gases such as hydrogen.

These conditions are reversed by just restricting the lactose rich food to the patients. However, milk is one of the balanced diets which contain essential nutrients such as proteins, vitamins, minerals for the vital functions of the body. Hence the present investigation has been undertaken to find out the lactose status of dairy products and to evaluate the activity of herbal extract for the possible breakdown of the complex sugar i.e. lactose into smaller units so as to assist the process of digestion.

Factors Affecting Milk Fat Content

Breed/Genetics

Between and within breeds, fat varies the most and lactose the least (Woodford et al., 1986). Gaunt (1980) reported cattle in the United States tend to have the lowest percentage of milk fat. This may be partly because of environmental factors, but some genetic variation within a breed in different countries must exist.

The repeatability from one lactation to another for the percentage of constituents in milk is quite high, an average of 0.67 (Gaunt, 1980). Repeatability of milk fat percentage for Holsteins is 0.76. Other breeds appear to have a similar repeatability.

Jerseys have the highest heritability for milk fat percentage (0.71), with other breeds ranging from 0.51 to 0.57. The small variation between ratios of one milk constituent to another, particularly fat to protein, suggests little hope for drastic changes in milk yield and milk composition (Gaunt, 1973; Wilcox, 1978). Heritabilities of solids-not-fat (SNF) to fat and protein to fat ratios are highest for Ayrshire followed by Jersey, Guernsey, Brown Swiss, and Holstein. Differences in heritabilities of breeds other than Holstein may be overestimated because of a small sample population.

Genetic correlations between milk composition percentages are high and positive, averaging 0.74. However, milk yield and composition percentages are negatively correlated, -0.3 for milk yield and fat percentage (Gaunt, 1980). Thus, it is very difficult to improve milk yield and milk percentage composition simultaneously.

Selection in Holstein cattle for the single trait of milk fat percentage would decrease milk yield by 287 pounds but increase fat percentage by 0.19 percent per generation. Selection for milk yield only increases milk yield by 607 pounds and decreases fat percentage by 0.036 percent.



Selection for milk fat yield is the most effective method for increasing fat percentage (+ 0.058 percent) and milk yield (+443 pounds) (Gaunt, 1980).

Environment/Man agement

A decrease in milk fat percentage of 0.2 percent over five lactations has been reported by Rogers and Stewart (1982). Fat yields would be expected to increase, since the increase in milk yields with age more than offsets the drop in fat percentage.

Milk fat percentages vary with stage of lactation. The highest percentages are usually found in colostrum, followed by a decline during the first 2 months of lactation, then a slow increase as lactation progresses. Davies et al. (1983) reported distinct changes in the fatty acid content of milk over the lactation cycle. During the first half, the proportions of short- and intermediate-chain fatty acids increase, and the proportion of long-chain fatty acids decreases. No further changes occur during the last half of lactation. Some of these changes are influenced by environment, diet, and rates of fatty acid synthesis in the mammary gland.

Seasonal variations in milk fat percentages are well recognized, with summer months averaging 0.4 percentage units less than winter months (Jenness, 1985). The higher environmental temperatures during the summer also affect milk fatty acid composition. Milk fat in the summer tends to be lower in palmitic acid relative to stearic and octadecanoic acids than milk fat from the same cows during the winter (Christie, 1979). Some of the changes in milk fat percentage and composition with temperature change can be related to changes in blood plasma lipids, but these observations are also confounded by dietary changes. Milam et al. (1986) observed no change in milk fat percentage when heat-stressed cows were given water at 10 or 28°C.

The fat percentage of milk increases continuously during the milking process, with the lowest fat milk drawn first and the highest fat milk drawn last. The increase in fat percentage throughout the milking process is due to the clustering of fat globules trapped in the alveoli (Jenness, 1985). Thus, if cows are not milked out completely, fat percentage will be lower than normal, but, at the next milking, fat content will be higher than normal. Furthermore, when milking intervals are unequal, the highest fat percentage is obtained after the shortest interval (Wheelock, 1980). Milk fatty acid composition is not affected by milking interval or time of day milking (Christie, 1979). The effect of milking three versus two times a day on milk fat percentage has varied, with some researchers reporting no change (Amos et al., 1985; DePeters et al., 1985; Gisi et al., 1986) and others reporting decreases (Allen et al., 1986; Gisi et al., 1986).

Health/Physiology

Mastitis (inflammation of the udder) generally causes a decline in milk fat percentage and a change in milk fat composition (Kitchen, 1981; Needs and Anderson, 1984; Schultz, 1977). The decrease in fat percentage, however, is less (about 10 percent) than that observed for lactose or casein (about 15 percent). Reported changes in milk fat composition from mastitis have varied.



There is general agreement on increases in amounts of free fatty acids and short-chain fatty acids, but both increases (Needs and Anderson, 1984) and decreases (Kitchen, 1981; Schultz, 1977) in phospholipid and long-chain fatty acids have been reported.

The effects of hormones on milk fat percentage are not well known (Bauman and Elliot, 1983; Tucker, 1985). It has been demonstrated that adrenaline and noradrenaline increase lipolytic activity in adipose tissue, but their effect on milk fat is unknown. Administration of exogenous growth hormone has resulted both in no change (Bauman et al., 1985; Peel et al., 1985) and in changes (Eppard et al., 1985) in milk fat percentage and composition. At low doses (5 and 10 IU/day), growth hormone lowered fat percentage with no change in fat composition, but at high doses (50 and 100 IU/day), milk fat percentage was increased and milk fat contained more endogenous fatty acids (Eppard et al., 1985). Growth hormone affected both synthesis of fatty acids in the mammary gland and uptake of preformed fatty acids from the blood, depending on dose level and energy balance of the cow. Sutton (1980) reported that the use of thyro protein, 1,3-butanediol, and glucocorticoids have generally not increased milk fat percentage.

Nutrition

Diets for today's high-producing dairy cows are typically higher in energy from readily fermentable carbohydrates than fats. Feeding of these diets often causes a condition known as low-milk-fat syndrome. Characteristics of low-milk-fat syndrome are a reduction in milk fat percentage (as much as 60 percent) and changes in milk fat composition (an increase in C_{18} polyunsaturated and monounsaturated acids and decreases in C_{160} and C_{180} fatty acids) (Banks et al., 1983; Christie, 1979). Causes of low-milk-fat syndrome probably involve both an alteration in rumen fermentation and availability of endogenous fatty acid sources (Christie, 1979). Feeding of readily fermentable carbohydrates depresses fiber digestion and pH in the rumen and thus decreases acetic and butyric acid production and increases propionic acid production. Increased propionic acid concentrations in the rumen lead to increased lactic acid and glucose production, which, in turn, stimulates insulin production, reducing free fatty acid serived from rumen fermentation, long-chain fatty acids of dietary origin, and acetic acid and long-chain fatty acids of dietary origin, and acetic acid and long-chain fatty acids from endogenous sources) can be affected by diet through changes in rumen fermentation or addition of fats for direct absorption and inclusion into milk fat.

Rumen Fermentation. Milk fat percentage is related positively to rumen molar percentages of acetic and butyric acids and negatively to that of propionic acid. Davis (1978) reported that rumen molar percentage of propionate must be above 25 before a highly significant negative relationship between milk fat percentage and propionate exists. Sutton (1980) estimated that 60 percent of the variations observed in milk fat percentage can be accounted for by changes in the molar proportion of propionate in the rumen.

A positive relationship exists between the molar ratio of acetate to propionate and milk fat percentage. A linear increase in milk fat percentage occurs as the ratio of acetate to propionate



increases up to 2.2 (Davis, 1978). Above a ratio of 2.2 there is little change in milk fat percentage. Thus, diets that increase propionate production have the greatest effect on milk fat percentage.

Numerous dietary factors affect rumen fermentation (Sutton, 1980). Those most commonly associated with changes in the acetate to propionate ratio are forage to concentrate ratio, type of carbohydrate in the diet, physical form of the diet, processing of ingredients, additives, and the frequency and method of offering feed. The following discussion summarizes the influence of these factors on rumen fermentation, acetate to propionate ratio, and change in milk fat percentage.

The general effect of decreasing the forage to concentrate ratio on rumen fermentation is to decrease pH, increase propionic acid production, and reduce fiber digestion. Thus, as forage declines, milk fat percentage falls proportionately; however, milk fat yields may increase (Sutton, 1980). The critical forage to concentrate ratio appears to be about 40:60, beyond which additional concentrate drastically lowers milk fat percentage (Coppock, 1985; Sutton, 1985). However, Sutton (1980) reported that the actual level of forage needed in a diet to maintain normal milk fat percentage may be affected by total feed intake. At high levels of intake, more forage is needed than at low-intake levels to maintain the same milk fat percentage. Recent work by Shaver et al. (1986) has shown similar results, with milk fat percentages being higher in milk from cows fed a 60:40 forage to grain diet at 2.93 percent of body weight than at 3.75 percent of body weight. Declines in milk fat percentage with high-grain feeding are accompanied by a change in milk fatty acid composition from saturated fatty acids to more unsaturated acids, especially those containing 16 carbons or less (Banks et al., 1983; Sutton, 1980).

The type of forage and its effect on milk fat percentage are influenced by forage particle size, maturity, and fiber content of the forage. It has been known for a while that finely ground forages reduce milk fat percentage. Finely ground forages apparently result in higher levels of propionate being produced during rumen fermentation than forages of adequate particle size (Sutton, 1980). Recent work by Woodford et al. (1986) has shown that a mean forage particle length of 0.64 cm or more is needed to keep rumen molar percentage of propionate below 25 and milk fat above 3.6 percent. Mertens (1985) recommended a minimum of 28 percent neutral detergent fiber and about 18 percent acid detergent fiber in diets to maximize milk production and fat percentage. The daily amount of neutral detergent fiber needed was estimated to be 1.2 percent of body weight.

Stage of forage maturity is an important factor in the supply of adequate fiber in the diet. More immature alfalfa hay was required in the diet to obtain maximum production of 4 percent fatcorrected milk than when mid- or late-bloom alfalfa hay was fed (Kawas et al., 1983). Recent work (Hansen et al., 1984) has shown that an interaction between forage species and concentrate level in the diet affects milk fat percentage. Bromegrass supported a higher milk fat percentage at higher concentrate feeding than did alfalfa. No difference between the two forage sources was observed at lower concentrate levels.



Carbohydrate source can influence rumen fermentation and consequently milk fat percentage. Sutton (1985) reported that the lower ruminal degradability of corn compared with that of barley would result in the production of milk with a higher fat percentage. Recent work (DePeters and Taylor, 1985) has confirmed that barley-based concentrates tend to depress fiber digestibility, resulting in lower ruminal acetate to propionate ratios and lower milk fat percentages than those with corn-based concentrates. The higher digestion of barley in the rumen produces more propionate and results in less starch being presented to the lower digestive tract for conversion to glucose than with corn. However, the increased production of propionate in the rumen from barley appeared to stimulate milk yield more than glucose derived directly from corn in the lower digestive tract. The mechanism(s) by which these two differences in nutrient supply affect milk fat is not well known. Processing of grains such as grinding, rolling, heating, steam flaking, and pelleting increases digestion of the starch in the rumen and produces effects similar to those reported above for barley (Sutton, 1980).

Increasing butyric acid production in the rumen should also help to maintain or increase milk fat percentages. Sutton (1980) suggested that beet pulp is a promoter of butyric acid production in the rumen. Other carbohydrates such as whey (Casper and Schingoethe, 1986; Schingoethe, 1976), sucrose, and lactose (Sutton, 1980) have been evaluated as sources of soluble carbohydrate to prevent milk fat depression.

The pattern of feeding, often referred to as feeding strategy, was found to have little if any benefit in terms of increasing milk fat percentage under normal conditions (Linn and Otterby, 1984). However, under feeding regimes where fat-depressing conditions are likely, increasing the frequency of offering concentrates to six or more times per day appears to stabilize the rumen environment (Bragg et al., 1986) and increase milk fat percentage (Sutton, 1980, 1985).

The mixing of all feed ingredients before feeding does not affect milk fat yield or percentage any differently than if the ingredients were fed separately (Holter et al., 1977; Marshall and Voigt, 1975; Owen, 1981).

Thomas and Chamberlain (1984) summarized the effects of infusion of specific nutrients into cows on changes in milk constituents. Intraruminal infusions of acetic acid consistently increase milk yield, lactose yield, and milk fat yield, whereas infusions of propionate reduce milk fat yield. Glucose infusions, either intraabomasal or intravenous, increase milk yield and decrease milk constituent percentages. Infusions of protein or amino acids (Schwab et al., 1976) have had variable or no effect on milk fat percentage.

The effects of dietary protein on milk fat percentage are variable but generally small when diets within normally accepted ranges of nutrients have been fed (Sutton, 1980; Thomas and Chamberlain, 1984). Changes in fat percentage result from changes in milk yield rather than from a direct effect of dietary protein source or amount. Insufficient amounts of rumen-degradable protein may lower milk fat percentage because of a lack of ruminal ammonia for optimal microbial digestion of fiber and other feed-stuffs.



Additives such as buffers and methionine hydroxy analog have been used to promote increases in milk fat percentage. Cows in early lactation fed high-concentrate diets were shown to benefit from the inclusion of the methionine hydroxy analog in their rations (Lundquist et al., 1983). Feeding of 25 grams of methionine hydroxy analog daily during the first 120 days of lactation increased milk fat 0.35 percentage units.

Buffers are compounds used to raise rumen pH through the neutralization of volatile fatty acids. However, other modes of action have been indicated for the group of compounds commonly alluded to as buffers (sodium bicarbonate, potassium bicarbonate, limestone, magnesium oxide, and bentonite) (Chalupa and Schneider, 1985). In general, the bicarbonates have been effective in maintaining or increasing milk fat percentages of cows fed high-grain diets, especially when corn silage was the main forage source (Chalupa and Schneider, 1985; Davis, 1978; Sutton, 1980). Magnesium oxide has also been shown to help prevent milk fat percentage depression; however, it appears that the mechanism of action is through transfer of lipid into the mammary gland from blood rather than through a change in rumen fermentation (Chalupa and Schneider, 1985).

A dded fats. Dietary fats can alter milk fat composition in a number of ways (Christie, 1979). One route is for fatty acids to be unaltered during digestion and absorption and therefore appear in milk fat directly. Another route is for the rumen microorganisms to hydrogenate the fatty acid, which can then appear in milk fat in this form or be further modified by desaturation before appearing in milk fat. Dietary fatty acids can appear in milk fat in the same form in which they were fed or be completely changed to another form before entering milk. In addition, the amount of particular fatty acids in the diet can alter lipid metabolism in the animal through mammary gland uptake problems or enzyme inhibitions. Dietary long-chain fatty acids can affect rumen fermentation and thus alter the amount of volatile fatty acids (acetic, propionic, and butyric acids) available for fat synthesis in the mammary, gland.

The use of fats and oils in the diets of dairy cows has received considerable attention (Fogerty and Johnson, 1980; Linn, 1983; Palmquist and Jenkins, 1980; Storry, 1980; Storry and Brumby, 1980). Numerous lipid sources, from natural to manufactured, have been evaluated. Their effects on milk yield and composition depend on type of fat, characteristics of the diet into which they are incorporated, rate and form fed, and method of feeding. Only a brief summary of changes in milk fat percentage and composition is reported here.

The changes in milk fat percentage and composition observed with the use of fat in diets of dairy cows are a reflection of the change in output of different fatty acids from the mammary gland; shortand medium-chain fatty acids (C_4 to C_{14}) are synthesized in the mammary gland, the C_{18} fatty acids come from the diet, and the C_{16} fatty acids come from both synthesis and dietary sources. Although dietary fats and oils may alter milk fat composition, the output of total milk fat depends on the balance of increased dietary transfer and decreased synthesis. However, there is probably a minimum content of short-chain fatty acids necessary to maintain melting points at body temperatures (Christie, 1979).



Both protected and unprotected fats and oils have been fed to dairy cows. Some of the unprotected fat or oil sources reported in the literature are tallow, yellow grease, vegetable oils, blends of animal-vegetable fats, and whole oilseeds (soybeans, sunflowers, cottonseed, and rapeseed). The common protected fat sources, so called because they are unavailable in the rumen and therefore do not alter rumen fermentation, fed are tallow and vegetable oils. Common methods of protection are formaldehyde-protein coating (Storry and Brumby, 1980) and formation of insoluble calcium salts of the fat (Jenkins and Palmquist, 1984).

In general, the addition of unprotected fat to dairy diets results in variable effects on milk yield and milk fat composition. The addition of fats, oils, or long-chain fatty acids depresses the synthesis of C_4 to C_{16} fatty acids in the mammary gland. This most likely results from an alteration in rumen fermentation rather than an inhibition of mammary gland acetyl-CoA carboxylase activity (Banks et al., 1983; Storry, 1980; Thomas, 1980). The effect on rumen fermentation is most pronounced with unsaturated fatty acid feeding. Long-chain fatty acid sources (more than 20 carbons) such as fish oils and Seterculia seed fats have a specific inhibitory action on the uptake of preformed fatty acids by the mammary gland. The changes in milk fat composition that occur with fat feeding are predominantly in the triglyceride fraction, with very little change occurring in the phospholipid and fat membrane fractions (Storry, 1980).

Protected polyunsaturated fatty acids appear to be the most promising for consistently increasing milk fat percentage and altering milk fat composition. Protected oil-seeds or oils rich in linoleic acid (sunflower, corn, and soybean) produce large, rapid increases in the linoleic acid content of milk fat when fed. The increases in linoleic acid content are generally associated with declines in myristic, palmitic, and oleic acids. Transfer of linoleic acid from protected supplements to milk is reported to be between 20 and 40 percent (Christie, 1979; Fogerty and Johnson, 1980).

Feeding of protected saturated fats, the most common source being tallow, generally invokes the same response in increase of milk fat percentage as feeding of protected polyunsaturated fats. However, protected hydrogenated soybean oil has decreased the milk fat percentage (Banks et al., 1983). Protected tallow increases the amounts of C₄, C₁₆₁, C₁₈₀, and C₁₈₁ fatty acids found in milk fat (Christie, 1979). Similar results were reported for unprotected tallow.

Protein

The total (crude) protein content of milk is determined by analyzing milk for nitrogen and multiplying by a factor of 6.38. The total protein percentage of milk is generally considered to be about 3.5, of which 94 to 95 percent is in the form of true protein (Davies et al., 1983; Jenness, 1985). Casein accounts for approximately 80 percent of the true protein, and milk serum or whey proteins account for about 20 percent. Urea is the largest single nonprotein nitrogen (NPN) component, accounting for approximately 50 percent of the total NPN (Wolf schoon-Pombo and Klostermeyer, 1981).



Milk proteins fall into several families of polypeptide chains, for which a systematic nomenclature system has been defined (Eigel et al., 1984). Casein proteins are characterized by ester-bound phosphate, high proline contents, and few or no cysteine residues and are precipitable from milk at pH 4.6 and 20°C. The main casein types in milk are alpha-, beta-, gamma-, and kappa-caseins. Whey proteins are distinguished from casein by remaining in solution upon precipitation of casein proteins. The major whey proteins are beta-lactoglobulin and alpha-lactalbumin. Serum albumin, immunoglobulins, proteose peptones, lactoferrin, and transferrin represent a smaller proportion of the whey protein fraction (Davies et al., 1983; Jenness, 1985; Kuzdzal-Savoie et al., 1980).

Biosynthesis

The synthesis of milk proteins has been extensively reviewed (Larson, 1979, 1985; Mercier and Gaye, 1083). In general, protein synthesis in mammary alveolar cells is similar to other protein synthesis systems in which DNA controls protein synthesis. Messenger RNA carries the encoded DNA message from the nucleus to the ribosomes located in the rough endoplasmic reticulum (RER) and cytoplasm. Ribosomes are composed of ribosomal RNA and several proteins combined into a ribonucleoprotein complex, which, in conjunction with transfer RNA, combines amino acids into peptide chains. As the polypeptide chains are elongated to form proteins, they pass out of the RER, through the lumen, and into the region of the Golgi apparatus where they accumulate and polymerize into different milk protein molecules. Casein must be phosphorylated, bound with calcium, and stabilized by calcium phosphate linkages and other ionic bonds before being released from the vesicles. The presence of alpha-lactalbumin in the region of the Golgi apparatus promotes synthesis of lactose. The secretory vesicles containing essentially nonfat milk constituents leave the cell by moving to the apical surface and fusing with the plasma membrane and discharging the vesicular contents into the cell lumen.

Most of the proteins present in milk are synthesized in the mammary gland, although some immunoglobulins and albumins are transferred from the blood (Larson, 1979). Blood leukocytes can also cross mammary barriers either by passing between secretory cells or by pushing secretory cells directly into the lumen. Urea diffuses freely across mammary cells, so there is a high correlation between blood plasma and milk urea concentrations (Thomas, 1980).

The synthesis of milk protein requires that both essential and nonessential amino acids be supplied to the mammary gland (Clark et al., 1978; Mepham, 1982). Uptake of free amino acids from the blood by the mammary gland can occur via several transport systems (Baumrucker, 1985). Mepham (1982) has classified essential and nonessential amino acids into three groups according to uptake by the mammary gland. Group I essential amino acids (methionine, histidine, phenylalanine, tyrosine, and tryptophan) are taken up in amounts just sufficient to meet milk protein synthesis needs. Group II essential amino acids (valine, leucine, isoleucine, arginine, lysine, and threonine) are taken up in excess. However, some data (Thomas, 1983) suggest that lysine and possibly leucine, isoleucine, and threonine should also be included in group I. Group III is the nonessential amino acids. The amounts taken up vary with animal, time, and availability. In addition to free amino acid uptake from blood, there is evidence that red



blood cells and the recycling of amino acids also contribute to the cellular amino acid pool (Baumrucker, 1985). Breakdown of red blood cell glutathionine can make a significant contribution to the amount of cysteine, glycine, and glutamic acid available in the cell. Recycling of casein proteins is reported to account for at least 7 percent of the protein synthetic capacity in the mammary gland.

Experimental:

In the preliminary analysis, different milk and milk products were tested for their lactose content separately using spectrophotometer at 540nm by following the methodology prescribed by Nickersan et al., (1976). From that a milk sample was chosen and mixed with the crude herbal extract such as ginger, tea, coffee, coriander and lactose level was estimated. 8g/ml of sample was taken and 1 ml of ZAPT (Zinc acetate phosphor tungestic acid) was added with the sample. This mixture was made into 10ml with distilled water and the content was filtered after 10 minutes using Whatmann no. 1 filter paper. With 0.5 ml of the filtrate, 0.5 ml of NaOH solution was added and diluted to 10 ml with distilled water and the mixture was filtered. 5 ml of this filtrate was diluted to 10 ml using distilled water. 5 ml of the above filtrate was mixed with 5 ml of glycine NaOH buffer, 0.5 ml of methyl amine solution and 0.5 ml of sodium sulphite. The content was mixed thoroughly and heated in water bath at 65° C for 25 minutes and cooled in ice for 2 minutes to stop the reaction. Absorbance was read at 540nm using spectrophotometer. A standard curve was drawn by plotting adsorbance against concentration of lactose and lactose level was determined separately for all samples.



Table 1: Reduction of Lactose content

Particulars	В	S 1	S2	S 3	S4	S5	T1	T2	T3	T4	T5	AD1	AD2	AD3	AD4
Volume of working standard (ml)		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Concentration of working standard (mg)		0.5	0.7 5	1.0 0	1.2 5	1.5									
Volume of Buffer (ml)		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Volume of methylamine solution (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Volume of sodium sulphite (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mix well and heat all the tubes in thermostatically controlled water bath at $65^{\circ}C$ for 25 minutes and cool immediately in ice water bath for 2 minutes to stop the reaction.															
Volume of distill water (ml)	10. 0														
Optical density at 540 nm	0.0 0	0.1 2	0.2 3	0.3 2	0.3 8	0.4 6	0.1 1	0.4 0	0.5 0	0.5 0	0.6 0	0.10	0.10	0.15	0.37
Lactose content (ppm)							22. 9	65. 7	81. 5	81. 5	97. 8	20.0	20.0	31.2 5	60.3

Results and Discussion

Among the tested herbal additives, ginger and tea extracts were found to be more active towards the reduction of lactose content of Amulya milk from the initial level of 65.7 ppm to 20 ppm (Table 1) and followed by coffee extract (31.2ppm). As described by Gekas and Lopes the hydrolysis of lactose could be prevented by processing of the milk and milk products including heating strategies. Most of the milk and milk products are coming to the market after such treatments. Hence the probability of lactose catabolism will get deprived. Their observations were close standing with the results of the present study. Stephenson et al. have analyzed the



level of severity of abdominal disorders in accordance with the lactose level in blood. Severity was more frequent in lactose intolerant than in lactose tolerant ones after the consumption of large quantity of milk and milk products. The concentration of lactose in the treated milk samples higher lactase production herbal additives could have played an indirect role to elevate the performance of the respective glands or cells.

Conclusion

It is concluded that the lactose content was well reduced in the commercial milk sample by adding the herbal additives such as ginger and tea extracts. The persons suffering from lactose intolerance are hence advised to take milk samples along with herbal additives thereby avoid the discomfort conditions arising due to lactose intolerance. Further research on this may lead to the discovery of a new more potent bioactive principle to suppress the lactose content in the milk thereby solving the permanent problem of lactose intolerance.

Reference:

- Danesh K Chitkara, Robert K Montgomery. Richard N Grand, Hands A Buller. Lactose Intolerance.
- V. Gekas; M. Lopez-Leiva, Hydrolysis of lactose: a literature review. Process Biochemistry. Vol. 20. No. 1, pp 2-12 (1999).
- 3. Lehninger's Principles of Biochemistry. Pp 285, 420, 686.
- L.S. Stephenson and M.C. Latham. Lactose intolerance and milk consumption: the relation of tolerance to symptoms; Graduate school of Nutrition, Cornell University, Ithaca, New York.
- 5. F.L. Saurez, D.A. Savaiano, M.D. Levit. The treatment of lactose intolerance-Department of Food Science and Nutrition, University of Minnesota, Minneapolis, USA.
- 6. Ambiga Shanmugam. Fundamentals of Pharmacy- 7th edition p 29 (2008)
- H.A. Biller and R J Grand Lactose intolerance- Annual review of Medicine, Vol 41, 141-148 (2010).



- Dallas M Swallow. Genetics of Lactase persistence and lactose intolerance. Galton lab., Department of Biology.
- 9. Harper's Biochemistry, 24th edition Prentice Hall International, pp 135, 165.