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RESEARCH ARTICLE

Development of a technique to detect the presence of cow milk in goat milk

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Abstract: Adulteration of Goat milk in terms of mixing with Cow milk has emerged to be a serious issue in the recent years. The higher price and nutritional value coupled with limited availability of the former serves as the driving factor for the mixing of relatively cheaper, easily available cow milk to goat milk. The current available analytical techniques for the detection of presence of cow milk in goat milk are highly sophisticated, complex and time consuming which cannot be adopted at the basic level by private goat milk producers. So, the development of a simple rapid laboratory technique for the same which can be applied at the farm and society level is a need of the hour. This study was conducted using pure goat milk, pure cow milk and cow milk mixed with goat milk at different proportions of 25%, 50 % and 75%. The physico chemical and compositional properties of samples were analysed and a significant variation between the samples was observed in terms of fat and chloride content. The ethanol stability was checked for the samples and found out that goat milk has lower ethanol stability compared to bovine milk and it improved by addition of cow milk. A blue colour which varies proportionately in intensity from light blue for pure goat milk to dark purple by increased addition of cow milk was observed by addition of Bromocresol purple and was verified using Hunterlab colorimeter. The addition of Seliwanoff' s reagent to the above said samples led to the development of coagulum with significant differences in appearance such as a completely dispersed coagulum in case of goat milk and a clearly settled coagulum on top portion for cow milk. The rate of settlement of coagulum to the top increases with increase in content of cow milk in goat milk. The above stated differences may be considered as the basis for detection of presence of cow milk in goat milk. Further the Scanning Electron Microscopic study of coagulum was carried out and fatty acid profile of samples was analysed using GC-MS, which finally confirmed our results. This led to the development of a rapid test for detection of inter- species adulteration of goat milk with cow milk.

Key words: Adulteration, Goat milk, Coagulum, Bromocresol purple, Seliwanoff's reagent

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Introduction

Food quality is a term that indicates the overall properties and attributes of a food article that are most acceptable to a consumer. It serves to be an integral and inevitable part of any food industry. The present era of globalization with improved standard of living marks the fact that the consumers are highly health conscious and they always pick out foods that are assured of high quality.Adulteration is a very serious persistent problem that is seen in the food industry which is highly detrimental to both the consumers as well as producers. If a particular product contains ingredients other than that specified in the food label, it can be regarded as a mode of adulteration.According to Food Safety and Standards Authority of India (FSSAI), adulteration of any food is defined as the addition or subtraction of any substance to or from food, so that the natural composition and quality of food substance is affected. Milk, a rapidly perishable product, is the easiest source of adulteration due to its compositional characteristics. The admixture of milk of one species with another without any specification also falls under this category. This can be at times so lethal because of the allergenicity of human beings to milks of specific species.

The worthiness of goat milk leads to its greater demand in the present market having a direct positive correlation with its higher price value. Despite all these facts the thought-provoking factor is the limited availability of goat milk over bovine milk. This coupled with the higher price of former paves the way for unscrupulous adulteration (Kang et al 2022). This type of

adulteration may lead to problems relating to health, religious, ethical or cultural objections and legal requirements. It is therefore desirable to confirm that goat milk offered for sale is free from admixtures of cow milk.

The private goat farmers constantly raise their suspicion of the practice of adding bovine milk in bulk goat milk by some producers. There is an alarming need for the development of a rapid laboratory test that would be able to detect the adulteration, as the currently available techniques are highly sophisticated, expensive and time consuming (Jing Yan Li et al 2023) The objective of this study to develop a rapid method for the detection of inter- species adulteration of goat milk with cow milk, which is of paramount importance and greatly beneficial for the layman.

Materials and methods

The initial step of procurement of raw materials was done by collecting pooled fresh goat milk and cow milk samples from University Goat & Livestock Farm and University Dairy Plant, Kerala Veterinary and Animal Sciences University, Kerala. As a preliminary trial the samples taken for analyses were pure goat milk, pure cow milk and cow milk mixed with goat milk at proportions of 5 %, 10 % and 25 %. Due toless sensitivity of the aforesaid proportions, further experiments were carried out with higher concentrations of 25%, 50 % and 75 % (a total of 5 samples).

Analysis of compositional and physico- chemical parameters

Various compositional and physico- chemical parameters of the samples were analysed with the primary aim of finding out a factor that exhibits a statistically significant difference between the samples. These parameters were analysed using the standard procedures prescribed by AOAC and BIS specifications.

Compositional parameters such as Fat (Gerber method), Protein (Kjeldahl method), Lactose (Lane- Eynon method), Chloride content (Mohr method) and Ash content (Gravimetric method) were analysed using standard method specified by BIS (IS: SP:18 [Part XI], 1981). Similarly physico- chemical aspects such as acidity and pH (IS: SP:18,1981), Electrical conductivity (Conductivitymeter, Systronics 306), Freezing point (IS1479 [Part 4: 2009), Refractive Index (AOAC 17thedn, 2000), Surface tension (AOAC 17thedn, 2000) and Specific gravity (IS 10083: 1982) were also analysed as part of the study.

Comparison of Ethanol stability

The differences in stability of the samples towards 70 % ethanol were analysed. A total of five samples (pure goat milk, pure cow milk, 25%, 50 % and 75 % mix of cow milk with goat milk) maintained at a temperature of 35- 40 °C were taken for the analysis. Five millilitreeach of thoroughly mixed milk samples and 70 %ethanol were taken in a test tube and shaken well. These were observed for any visible coagulation. The test samples were kept in a boiling water bath for about 3 minutes. The differences in coagulation as well as the separation of layers together with the nature of coagulum particles formed at the sides of the test tube were observed.

Reaction with Bromocresol purple reagent

Five millilitres of thoroughly mixed milk samples(pure goat milk, pure cow milk, 25 %t, 50 %and 75 %mix of cow milk with goat milk) maintained at a temperature of 35- 40 °C were taken in test tubes. Five millilitresof distilled water followed by 1 millilitre Bromocresol purple reagent was added and mixed well. The difference in the intensity of blue colour developed was noted and measured quantitatively by Hunterlab colorimeter.

Hunter Colorimeter

Colour of the samples was measured by reflectance spectroscopy technique using reflectance meter (color flex, Hunter lab Miniscan XE plus Spectrocolrimeter, Virginia, USA) with geometry of diffuse/8p (sphere- 8mm view) and an illuminant of D65/10p . Before the test, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer. The light source was dual beam xenon flash lamp. Data was received from the software in terms of L^* [Lightness, ranges 0 (black) to 100 (white)], a^* [Redness- positive, Grey- zero, Greenness- negative] and b^*[Yellowness- positive, Grey- zero, Blueness- negative] values of the International Colour System.

Reaction with Bromocresol purple and Seliwanoff's reagent

Seliwanoff's reagent was added to the samples mixed with Bromocresol purple solution. To the obtained blue colour solution 1millilitre of freshly prepared Seliwanoff's reagent (0.5 % resorcinol in 3N HCl) was added and properly mixed. These test tubes were kept in a boiling water bath for about 5 minutes. The appearance of the coagulum formed was observed for the distinctive features. The coagulum formed was later subjected to centrifugation in a laboratory centrifuge at 4000 rpm for 5 minutes to find out the variations in the amount of sediment obtained for each sample.

Other parameters like microscopic view of milk coagulum by Scanning Electron Microscopy (SEM) and fatty acid profile of milk samples using Gas Chromatography- Mass Spectrometry (GC-MS) were also studied.

Statistical Analysis

The results obtained from the analysis were verified using One way Analysis of Variance (ANOVA) with Duncan test at 5 percent level of significance and correlation coefficients statistically using SPSS (Statistical Packages for Software Solutions) software, Version 21.0 designed by IBM Company, USA and data were expressed as Mean ± Standard Error.

Results and Discussion

Comparison of compositional and physico- chemical parameters

The results for compositional and physico- chemical parameters of the samples are shown in Table 1 and Table 2 respectively.One way ANOVA analysis of the parameters by Duncan tests at 5 percent level of significance indicated that there was a statistically non-significant difference between the samples in these parameters with an exception of fat, chloride content and refractive index that differs significantly. Fat is one of the most variable components and has shown a statistically significant difference between each sample which may account for the change in breed of the animals. A decrease in fat content was observed when the quantity of cow milk mixed with goat milk increased. The detection limit when considering the case of variation in fat content can be taken as 25%. Pure goat milk exhibited significant difference in chloride content with 50 %mix, 75 %t mix and pure cow milk but non-significant difference with 25 %mix of cow milk. This implies that if cow milk is being mixed with pure goat milk, the minimum detection limit in terms of change in chloride content is found out to be 50%. The electrical conductivity of samples varied almost linearly with the level of chloride content with the highest for goat milk. Both the parameters decreased proportionately with an increase in the proportion of cow milk being mixed with goat milk. The next variable factor is refractive index in the case of which pure goat milk varied significantly with 50%, 75 % mix and pure cow milk.

Relation between chloride content and electrical conductivity

A positive correlation was observed between the chloride content (percent) and electrical conductivity (mS) of samples. There was a proportionate increase in conductivity with increase in chloride content with goat milk possessing the highest value.

The above stated parameters cannot be relied upon since these may vary according to breed, season, stage of lactation, udder infection, temperature etc.

Difference in Ethanol stability

An immediate coagulation was observed in case of pure goat milk with no coagulation at all for pure cow milk. As the quantity of cow milk mixed with goat milk increases the rate of coagulation was found to decrease. The minimum level of detection limit was found to be 50 percent. Decreasing order of rate of coagulation is: Pure goat milk> 25 %mix> 50 %mix> 75 %t mix> Pure cow milk (Figure 1).The rate of coagulation was higher for goat milk with 70 % ethanol when compared to cow milk which shows that the former had lower ethanol stability than the latter. When the quantity of cow milk admixture with goat milk increases the rate of coagulation decreased proportionally i.e., an increase in ethanol stability was observed. The higher rate of coagulation of goat milk was attributed to the lower content or absence of αs1 casein in it (Feligini et al. 2009). The αs1 casein fraction in milk has the ability to trap calcium ions and withdraws them from the proteolysis of k casein, thereby retards the rate of curd formation. Hence greater amount of free calcium remains unbound by virtue of the lesser quantity of casein present in goat milk.

Table 2: Change in physico- chemical parameters for pure goat milk, pure cow milk and admixture samples

Parameter	Goat milk	25% mix	50% mix	75% mix	Cow milk
Fat (%)	5.111 ± 0.355^{a}	4.811 ± 0.289^{c}	4.411 ± 0.169^{d}	4.011 ± 0.136^{e}	3.667 ± 0.194^{b}
Lactose (%)	5.022 ± 0.151^{ns}	4.975 ± 0.171^{ns}	4.922 ± 0.201^{ns}	4.809 ± 0.125^{ns}	4.813 ± 0.267^{ns}
Chloride (%)	0.183 ± 0.013^{a}	0.170 ± 0.013^{bc}	0.161 ± 0.014^{cd}	0.149 ± 0.010^{d}	0.141 ± 0.015^{ab}
Protein (%)	3.496	3.494	3.485	3.470	3.482
Ash (%)	0.840	0.796	0.772	0.763	0.778

Figures are Mean average values and Mean± Standard error, $^{a-e}$ figures in row bearing different superscripts differ significantly, ^{ns}- non significant

Table 2: Change in physico- chemical parameters for pure goat milk, pure cow milk and admixture samples

Parameter	Goat milk	25% mix	50% mix	75% mix	Cow milk
Acidity(%LA)	0.186 ± 0.022^{ns}	0.171 ± 0.016^{ns}	0.171 ± 0.016^{ns}	0.171 ± 0.016^{ns}	0.158 ± 0.008^{ns}
pH	6.528 ± 0.031^{ns}	6.547 ± 0.065^{ns}	6.547 ± 0.065^{ns}	6.547 ± 0.065^{ns}	6.608 ± 0.094^{ns}
Electrical conductivity (mS)	6.628 ± 0.301^{ns}	6.524 ± 0.309^{ns}	6.394 ± 0.307^{ns}	6.307 ± 0.332^{ns}	6.172 ± 0.303^{ns}
Freezing point (°C)	-0.557 ± 0.017^{ns}	-0.550 ± 0.010^{ns}	-0.540 ± 0.002^{ns}	-0.537 ± 0.003^{ns}	-0.530 ± 0.007^{ns}
Refractive index	1.353 ± 0.002^{a}	1.351 ± 0.001^{b}	1.351 ± 0.001^{b}	1.350 ± 0.001^{b}	1.350 ± 0.001^{ab}
Surface tension (N/m)	0.059 ± 0.007^{ns}	0.060 ± 0.010^{ns}	0.060 ± 0.010^{ns}	0.060 ± 0.004^{ns}	0.063 ± 0.004^{ns}
Specific gravity	1.034 ± 0.002^{ns}	1.033 ± 0.002^{ns}	1.032 ± 0.001^{ns}	1.031 ± 0.001^{ns}	1.031 ± 0.002^{ns}

Figures are Mean± Standard error, $^{a-e}$ figures in row bearing different superscripts differ significantly, ^{ns}- non significant

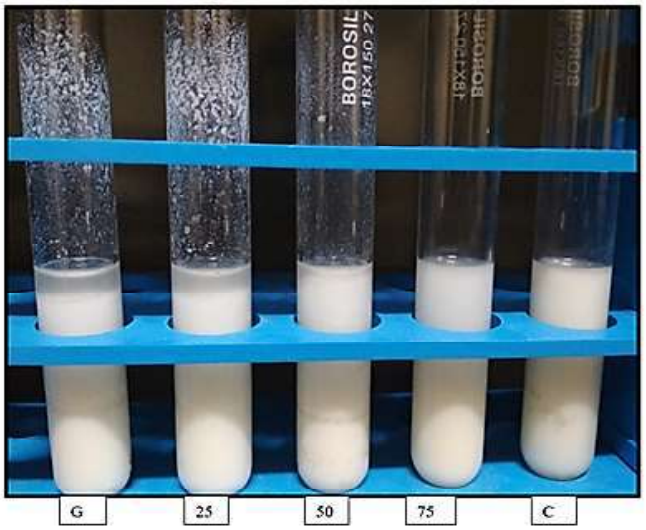

Fig. 1 Decreasing order of coagulation after addition of 70% ethanol

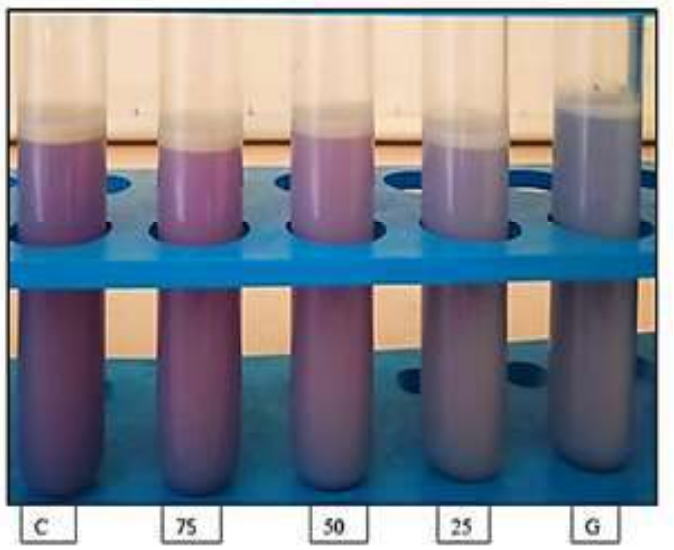

Fig. 2 Decreasing order of colour intensity after addition of Bromocresol purple reagent

The amount of casein, surface hydrophobicity, collision distance of proteins, size and integrity of micelles are some of the important factors affecting the coagulation properties of milk samples. The greater size of casein micelles causes shorter collision distance between the proteins that forms the basis for the reduced coagulation time of goat milk compared to cow milk (Bonomi et al. 1988).Moreover, surface hydrophobicity of goat milk is greater since it consists of β casein, the most hydrophobic of all the caseins as the major casein component (Mellema et al.1999).

Stocco (2018) found out that the fat content in milk has an effect on the coagulation characteristics. Better coagulation properties of goat milk may be attributed to its higher fat content as milk flocculation occurs due to the collision and aggregation of fat droplets. The fat globules that get entrapped in the casein network and thereby forming a surface coating, behaves to an extent like casein micelles and accelerates the kinetics of coagulation (Sweetsurand Muir1983).Studies conducted by Horne and Parker (1982) stated that the low ethanol stability of goat milk may be caused because of its lower micellar charge. The negative charge of protein gets neutralized by the more available free calcium (Ca^{2+}) in case of goat milk.

Difference in reaction with Bromocresol purple reagent

Goat milk and cow milk exhibited remarkable differences in their reaction with Bromocresol purple (BCP) reagent. Both the milks developed a purplish-blue colour when mixed with Bromocresol purple reagent, but the intensity of colour varied. A lighter colour was observed in case of pure goat milk and a relatively darker one for pure cow milk. The intensity of blue colour increases when the quantity of cow milk mixed with goat milk increases. The decreasing order of the samples according to the intensity of colour is: Pure cow milk > 75 %mix > 50 %mix > 25 % mix > Pure goat milk (Figure 2).

Bromocresol purple (BCP) or otherwise called 5', 5"- dibromo-o-cresolsulfophthalein is a dye of the triphenylmethane family. It is a commonly used pH indicator which is coloured yellow below pH 5.2 and purple above pH 6.8. Its structure changes with the pH with different colour for each structure. The chemical has a sulfonate structure in near neutral or alkaline pH that gives the solution a purple colour. It converts to a sultone (cyclic sulfonic ester) that colours the solution yellow as the pH decreases.The increase in pH to the alkaline range may be caused due to the addition of distilled water to the samples. This may enhance the identification of colour due to greater scattering of light. The indicator transfers a proton from it to water and thereby shifts the equilibrium to the side of the conjugate base and hence the development of purple colour (Pradeep and Dave 2013).

The lighter intensity of colour in case of pure goat milk may be due to the increased fat content as it may hinder the absorption of light by Bromo cresol purple. The intensity of colour increased with the quantity of cow milk being admixture with goat milk. Since cow milk has a lower fat content relative to goat milk, the former developed a comparatively intense purple colour. The increased colour intensity with increase in proportion of cow milk may be because of the decrease in fat content.Moreover, goat milk has a lower pH value compared to cow milk which may also contribute to the relatively less intense colour.

The colorimetric readings (L^*, a*, b*) confirmed the obtained results. From the results it is clear that since the intensity of blue colour is lower for pure goat milk, it has the highest L^* and lowest b^* value which implies that it is lighter in colour with reduced degree of blueness. Cow milk was more intense in colour with

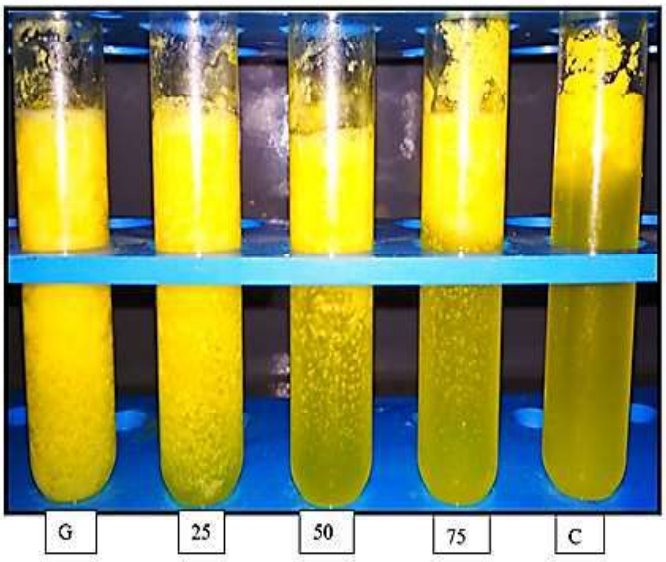

Fig. 3 Decreasing order of rate of coagulation after addition of Bromocresol purple and Seliwanoff's reagent

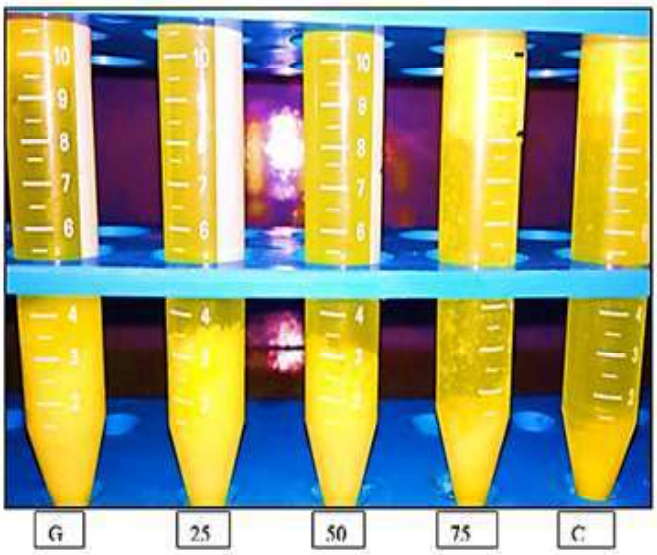

Fig. 4 Sediment formed after centrifugation of coagulum

maximum b^* value for it. A significant decrease and increase were observed in the L^* and b^* values respectively with the addition of cow milk to goat milk.

Difference in reaction with Bromocresol purple and Seliwanoff's reagent

The purplish-blue colour developed upon the addition of Bromocresol purple reagent changed into yellow colour when Seliwanoff's reagent was added to it. All the samples were almost the same in their appearance by visual observation before heating. Coagulation takes place in the samples once they are kept in boiling water for about 5 minutes. The nature and structure of the coagulum developed varied according to the samples. While a completely dispersed coagulum was observed in goat milk, pasty compact cement like coagulum which settles to the top developed in case of cow milk. The rate of settlement of coagulum to the top portion increases when the quantity of cow milk mixed with goat milk increases (Figure 3).

Seliwanoff's reagent is used in sugar test to differentiate between aldose and ketose sugars which consists of concentrated HCl and resorcinol as the major components. The addition of Seliwanoff's reagent causes the decrease in pH and at lower pH (below 5.2) value bromocresol purple developed yellow colour and higher acidity leads to the development of coagulum. The presence of resorcinol gives the solution a bright yellow colour. The smaller size of casein micelles in case of cow milk caused the entire coagulum to settle to the top. The relatively lower content of αs1 casein together with greater casein micelle size made the goat milk coagulum to get completely dispersed.

The amount of sediment produced when the coagulum was subjected to centrifugation at 4000 rpm for 5 minutes also varied for the samples (Figure 4). The coagulum does not completely settle down in case of pure cow milk with some part of it remaining at the top portion while a completely settled coagulum was observed in case of pure goat milk after centrifugation. The amount of settled coagulum decreased with the increase in quantity of cow milk .The smaller size of casein micelles in case of cow milk caused the entire coagulum to settle to the top. The relatively lower content of αs1 casein together with greater casein micelle size made the goat milk coagulum to get completely dispersed.

Difference in structure of milk coagulum

The coagulum developed in each sample after the addition of 70 %ethanol was viewed microscopically by means of Scanning Electron Microscopy (SEM). The difference in the structure and nature of coagulum observed under SEM is given in Plate 1, Plate 2 and Plate 3.

The SEM view of the coagulum of the samples indicated a near clear cut difference in their nature and structure. This may be attributed to their compositional variation such as mineral balance, protein contents etc. A dispersed less firm and non-pasty structure was developed in case of goat milk (Plate 1) while a pasty firm and compact cement like one in cow milk (Plate 2). The 50 %admixture of both goat milk and cow milk had a structure that lies in between both (Plate 3). The weaker scattered coagulum developed in goat milk may be due to the presence of large irregular void spaces in the protein matrix that leads to greater destruction and deformation of the coagulum structure.According to Park et al. (2007) goat milk forms a weaker

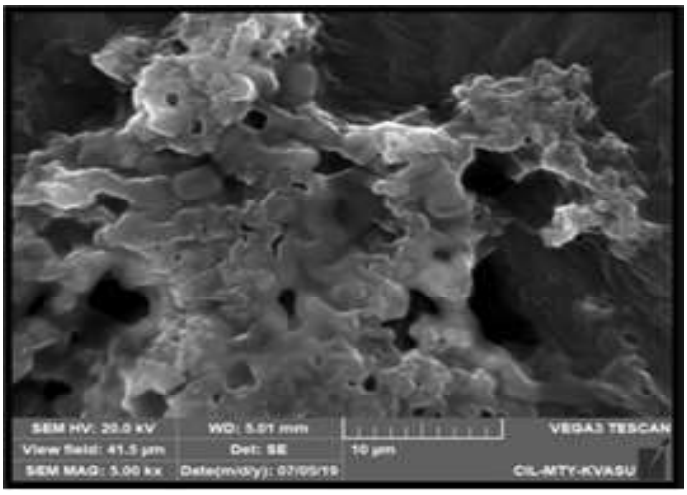

Plate 1: Coagulum of pure goat milk

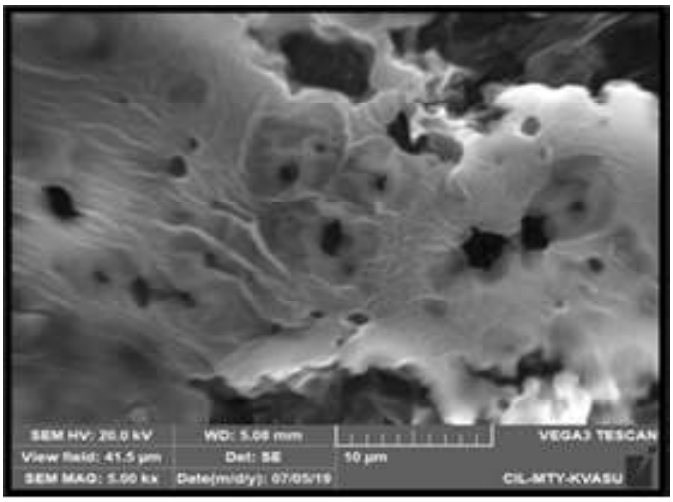

Plate 2: Coagulum of pure cow milk

texture of coagulum due to the lower concentration and ratio of casein fractions and relatively larger size of casein micelles compared to bovine milk. The former also consists of higher free calcium in the serum which may also serve to be a reason for its structure difference with cow milk.

Difference in Fatty acid profile

The variations in the contents of individual fatty acids in terms of fatty acid profile of pure goat milk, pure cow milk and a 50 %mix of both was found out using Gas Chromatography- Mass Spectrometry (GC- MS). The contents of short and medium chain fatty acids such as caproic, caprylic, capric and lauric acid is greater in goat milk whereas cow milk had higher contents of myristic, palmittic, palmitoleic and stearic acids(Vieitez et al. 2016).Butyric acid is present in more amounts in cow milk..The results were in accordance with the standard contents of fatty acids stated by Runowska et al (2013). A proportionate decrease in the short and medium chain fatty acids (C6: 0 caproic acid to C12: 0) present in goat milk was observed when cow milk being mixed with it at 50 percent level. Similarly, the level of long chain fatty acids increased in goat milk upon admixture. This could be used as a tool for indicating whether cow milk has been mixed with goat milk.

Conclusion

A blue colour which varies proportionately in intensity from light blue for pure goat milk to dark purple by increased addition of cow milk was developed by the addition of Bromocresol purple and the same was verified using colorimeter.The addition of Seliwanoffs reagent to the abovesaid samples led to the development of coagulum with significant differences in appearance such as a completely dispersed coaguilum in case of goat milk and a clearly settled coagulum on top portion for cow milk. The rate of settlement of coagulum to the top increases with increase in content of cow milk in goat milk. The above stated

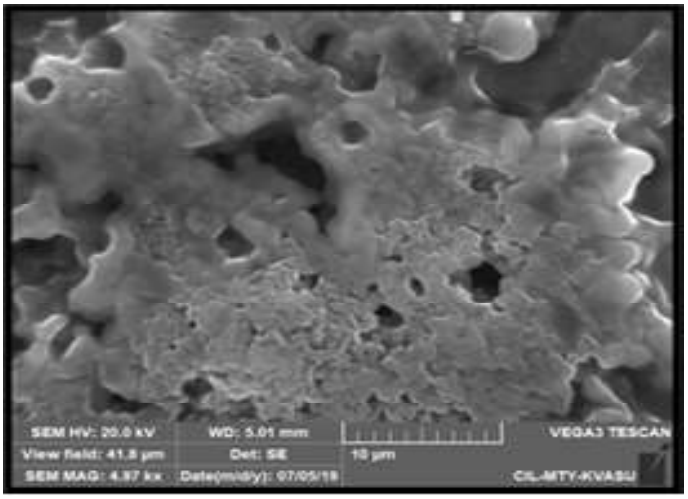

Plate 3: Coagulum of 50 percent mix

differences may be considered as the basis for detection of presence of cow milk in goat milk. Further the Scanning Electron Microscopic studies of coagulum was carried out and fatty acid profile of samples was analyzed using GC- MS. These data finally confirmed our results.

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