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Effect of ingredient variation on microbial acidification, susceptibility to syneresis, water holding capacity and viscosity of soy-peanut-cow milk yoghurt

Abstract

Acidification of milk by lactic acid bacteria enhances the aggregation of milk proteins to form yoghurt gels with enhanced texture, colour and viscosity. A three-component constrained mixture design was employed to develop 10 soy-peanut-cow milk (SPCM) formulations which were fermented with *Lactobacillus bulgaricus and Streptococcus thermophilus* (1:1) into soy-peanut-cow milk yoghurt (SPCY). The effect of ingredient variations on microbial acidification, colour, susceptibility to syneresis, water holding capacity and viscosity were determined. Titratable acidity increased with increasing cow milk content and trends in pH were contrary to titratable acidity. SPCY formulations were yellowish-white in colour. Yellowness and lightness increased with increasing soymilk content. Rheologically all products investigated were non-Newtonian and had better consistencies as cow milk content increased in samples and peanut milk content decreased. The water holding capacities of yoghurt samples increased with increasing soy milk content. Formulations without cow milk were the least susceptible to syneresis.

Keywords: evegetable milk, yoghurt, starter culture, viscosity, susceptibility to syneresis, souring

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Introduction

Yoghurt is a fermented milk product and has been noted to be the most widely consumed fermented dessert worldwide.[1] Traditionally, the product is prepared by fermenting cow milk with lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Acidification of milk by lactic acid bacteria enhances the aggregation of milk proteins to form a yoghurt gel. When milk is fermented and in the process gets acidified, the internal structural properties of casein miscells are disrupted.[2] Acid induced milk gels are formed by the aggregation of casein particles as the pH of milk decreases and caseins approach their isoelectric point (pH 4.6).[3] Milk-like beverages manufactured from legumes such as soya beans and peanut have also been noted as potential nutritional substitutes in cultures where cow milk is used.[4,5] Soy-peanut yoghurt is a composite fermented milk product developed by culturing milk extracts from soybeans and peanut with lactic acid producing bacteria. Since yoghurt is basically a protein gel formed as a result of the acidification of milk by starter bacteria mainly lactic acid bacteria, a strong soy protein-peanut protein- cow milk protein interaction could generate a stable milk gel. Yoghurt manufactured from a composite milk blend can offer a considerable appeal for a growing segment of consumers with certain dietary and health concern. The need to also exploit further sources of high quality food proteins and high energy foods has made it imperative to study the possibility of developing soy-peanut-cow milk yoghurt.

Fermentation processes have been utilized to improve the sensorial attributes and also to decrease the properties of undesirable compounds in products. Lactic acid fermentation has been reported as a means to reduce beany flavours and anti-nutritional factors, such as phytic acid in soy beans.[6] Aside fermentation, effect of soaking in reducing the apparent beany/nutty flavours of legumes have been studied.[7] In producing Soy-peanut-cow milk yoghurt, the study combined soaking of oilseeds in sodium bicarbonate solution, heat treatment and fermentation processes to further enhance the removal of undesirable components in oilseeds.[8]

Functional properties of food proteins are essential factors to consider in food processing or in the formulation of new food products.[9] Proteins impart desirable functional properties like water holding capacities, viscosity, emulsification, gelatin, foam formation and whipping capacity to food systems.[9,10] The functionality of soy and peanut protein in food systems is influenced by intrinsic factors of the protein and the presence of other components in the food environment.[10] Water holding capacity is a term that is frequently employed to describe the ability of a matrix of molecules to physically entrap large amounts of water in a manner that inhibits exudation.[11] WHC of a protein gel is a vital consideration in yoghurt manufacturing, because it is related to syneresis, which is due to the intrinsic instability of gels resulting in loss of water after some storage time.[12] Syneresis is a quality defect frequently faced in yoghurt manufacture. Less syneresis had been found in yoghurts produced from a mixture of skim milk and soymilk regardless of the type of starter culture utilized.[13] The water holding capacity of peanut milk yoghurt has also been observed to be significantly higher than that of cow milk yoghurt.[4] Incorporating soy and peanut proteins in yoghurt manufacture can limit syneresis and improve the water holding capacity of the product. Consumer acceptance of liquid based semisolid type foods is also dependent on the viscosity and consistency of the product; hence the viscosities of 10 soy-peanut-cow milk yoghurt formulations consisting of varying proportions of soy milk, peanut milk and cow milk were also studied.

A mixture design was applied in combining milk from three different sources. A mixture design was used for this study because components of a mixture are limited by an implicit constraint that the sum of all components must be-1 (100%). Components cannot be varied independently because by varying the percentage of one component, percentages of the other components change.[14] The composite milk blend was subsequently fermented into yoghurt. The study investigated the effect of ingredient variations on microbial acidification, colour, susceptibility to syneresis, water holding capacity and viscosity of soy-peanut-cow milk yoghurt (SPCY) formulations.

Materials and methods

Materials

Red-skinned peanut seeds (Chinese variety) and soya bean seeds (Jenguma variety) were obtained from a registered seed grower and care was taken to ensure that good quality and mould-free seeds were selected. The starter culture (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*) and cow milk used for study were obtained from Amrahia Dairy Farms, Amrahia in Accra, Ghana.

Milk preparation: Peanut milk and soy milk were prepared by modifying a method reported by Aidoo et al.[7] Sorted peanut seeds were blanched by submerging in boiling water (100°C) for 10minute to inactivate the enzyme lipoxygenase known for its ability to cause oxidation which leads to the production of beany flavour. The seeds were then de-skinned and weighed before being soaked in 2% NaHCO3 for 18hours. The de-skinned peanut kernels were washed with hot water (70°C). Soy beans were also steeped in boiling water for about 10 minutes, dehulled, weighed and then steeped in water for 16hrs followed by 2% NaHCO3 for 2hours. Soaking in NaHCO3 was to soften the seeds and also remove the beany flavour as much as possible. The beans were then washed in hot water. The dehulled peanut and soya beans were separately mixed with water in a ratio of 1:5 w/v and then milled to obtain the slurry.[4] The slurry was filtered to obtain a smooth, fine, homogenized milk. Cow milk was added to the prepared soy milk and peanut milk to obtain the soy-peanut-cow milk for the study.

Mixture design: Ten milk formulations were processed into yoghurt by mixing the three basic ingredients; peanut milk (PM), soy milk (SM) and cow milk (CM). The proportions of these ingredients were obtained using a three component constrained mixture design.[15] Using design of experiments software, Minitab version 14, a mixture design (centroid design) was used to obtain 10 design points from three components. The design was used to determine the optimum ratios of peanut milk, soy milk and cow milk that will yield the most acceptable product (Table 1).

Table 1 Design matrix for ingredient formulations using ratio of the SPCM mixtures

Formulation	Soy milk	Peanut milk	Cow milk
F1	0.60	0.40	0.00
F2	0.70	0.20	0.10
F3	0.63	0.33	0.03
F4	0.60	0.20	0.20
F5	0.80	0.20	0.00
F6	0.63	0.23	0.13
F7	0.70	0.30	0.00
F8	0.73	0.23	0.03
F9	0.60	0.30	0.10
F10	0.67	0.27	0.07

Yoghurt preparation:

Starter culture preparation: Freeze-dried yoghurt starter cultures of *S. thermophilus* and *L. bulgaricus* were obtained and revived separately in 12g/100 g sterilized milk broth and then transferred to soya-peanut-cow milk broth for yoghurt production.[4]

Preparation of soy-peanut-cow milk yoghurt: Soy-peanut-cow milk yoghurt was prepared by modifying a method reported earlier by Isanga & Zhang.[4] Each soya-peanut-cow milk formulation was mixed and warmed at 43°C for 30minutes. The milk was homogenized and pasteurized at 85°C for 30minutes. The pasteurized milk was cooled to 43°C in a water bath and then inoculated with 3ml starter culture (*L. bulgaricus* and *S. thermophilus*; 1:1) per 100ml milk. The mixture was incubated at 43°C for 3-4hours. At the end of the incubation period, the yoghurt was cooled and then transferred to a refrigerator at ~5°C where it was stored overnight prior to analysis.

Analytical methods

Physico-chemical properties

Non-volatile (titratable) acidity, pH and colour: Non-volatile (titratable) acidity was determined using AOAC method 947.05[16] by titration with 0.1N NaOH solution and expressed as percent lactic acid while the pH of the samples was measured using a pH meter (Hanna Instrument pH 210, Microprocessor pH meter, Duisburg, Germany). The colour of soy-peanut-cow milk yoghurt was determined using a colorimeter.[17,18]

Rheological characteristics

Apparent viscosity: The apparent viscosity and shear rate of the yoghurt was measured at 10°C using a Brook-field viscometer (Brook-field model LVDVI, AE42086, Springfield, MA, USA). The flow curves of the yoghurt formulations were obtained by varying the shear rate from 10 to 60s^{-1} and the corresponding viscosity values measured.[4]

Water holding capacity: The water holding capacity (WHC) of yoghurt was determined by a method reported by Harte and Barbosa-Canovas[19] with slight modifications. The yoghurt was subjected to 30-min centrifugation at 4000×g at a temperature of 10°C using a centrifuge. WHC of the samples were calculated using the following equation: WHC (%) = $(1- W_1/W_2) \times 100$

where: W_1=Weight of whey after centrifugation, W_2=Yoghurt weight.

Susceptibility to syneresis

The yoghurt susceptibility to syneresis (STS) was measured by placing 100ml of yoghurt sample on a filter paper placed on top of a funnel. After 6hours of drainage, the volume of the whey collected in a beaker was measured and used as an index of syneresis.[4] The following formula was used to calculate STS: STS (%)= $V_1/V_2 \times 100$, where: V_1=Volume of whey collected after drainage; V_2 =Volume of yoghurt sample.

Statistical analysis

Data obtained was analyzed using MINITAB and Statgraphics (Graphics Software System, STCC, Inc. U.S.A). Comparisons between the 10 Soy-peanut-cow milk yoghurt formulations were done using analysis of variance (ANOVA) with a probability, $p<0.05$. All treatments were conducted in duplicates. The analyses were conducted in triplicates and the mean values reported.

Results and discussion

pH and titratable acidity of SPCY formulations

pH values of SPCY formulations varied from 4.3 to 6.63 depending on the cow milk content of the sample. pH decreased with increasing cow milk content and increased with increasing soymilk/peanut milk (Figure 1). Yoghurt formulations with different soy milk and cow milk combinations have been observed to have pH values ranging from 3.30 to 4.50, with samples having increased soy milk proportion recording high pH values.[20] SPCY formulations which contained 10% to 20% cow milk recorded low pH values of 4.30 to 5.65; whereas samples with cow milk content of 0 to 3% recorded high pH values (5.86 to 6.63).

Titratable acidity of samples was contrary to pH values of formulations. Samples with low cow milk content (0 to 3%) had the least total acid content (0.07% to 0.18%); while those with high cow milk content (7% to 20%) had high total acidity within the range of 0.22% to 0.32%. Titratable acidity of samples increased with increasing cow milk content and decreased with increasing soy milk content (Figure 2). Since cow milk contains lactose which is an ideal substrate for yoghurt starter cultures, the microorganism efficiently utilized the lactose in cow milk to release more lactic acid which decreased the pH of soy-peanut-cow milk yoghurts with high cow milk content. pH and titratable acidity of all SPCY formulations and the control were significantly different (p<0.05). The titratable acidity observed for the control was 0.55%, significantly higher than that recorded for all 10 SPCY formulations.

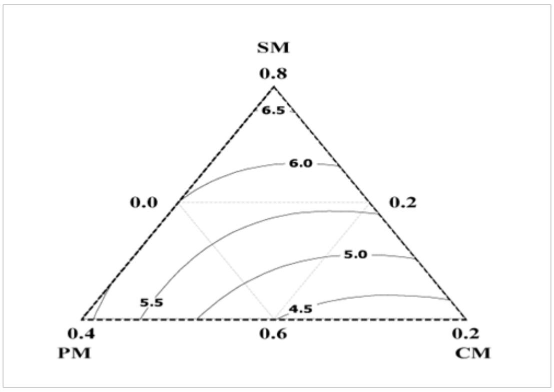

Figure 1 Mixture contour plot of pH of SPCY formulations.

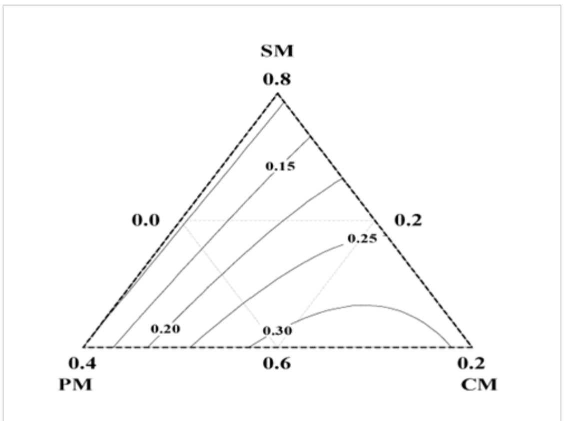

Figure 2 Mixture contour plot of titratable acidity of SPCY formulations.

Colour of SPCY formulations

The colour of a product is a critical sensory attribute which informs consumer acceptability of a product. The lightness L* of SPCY formulations varied from 65.47 to 70.92. The lightness (whiteness) increased as the proportion of soy milk in the mixture increased (Figure 3). b* value when positive signifies a yellowish colour coordinate. Results obtained for b* for all SPCY formulations were positive and fell within the range +11.69 to +13.01. Yellowness decreased as the proportion of soy milk decreased in the samples (Figure 4). a* indicates redness of samples and this increased in SPCY formulations as peanut milk in samples increased (Figure 5). a* values ranged from -2.38 to -1.81. SPCY formulations were generally yellowish-white in colour.

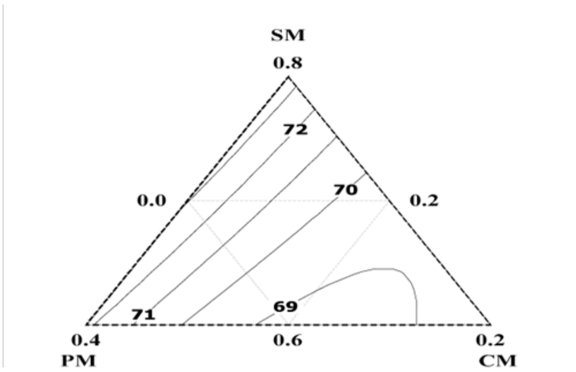

Figure 3 Mixture contour plot of L* values of SPCY formulations.

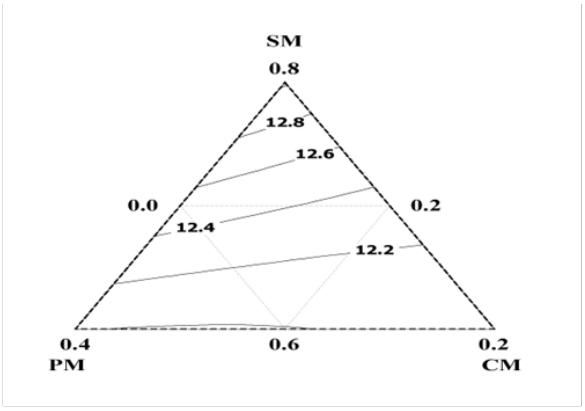

Figure 4 Mixture contour plot of b* values of SPCY formulations.

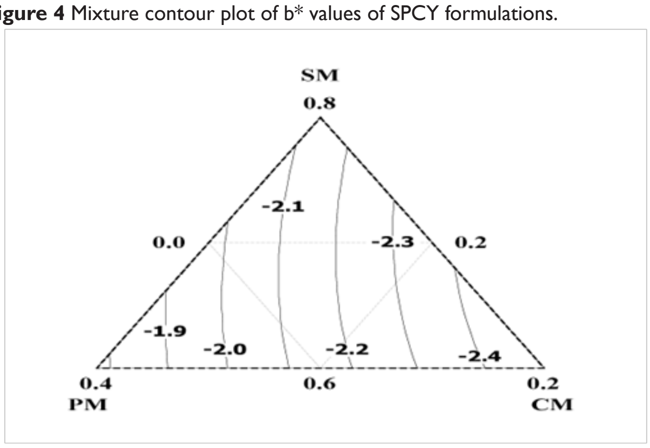

Figure 5 Mixture contour plot of a* values of SPCY formulations.

Apparent viscosity of SPCY formulations

All SPCY formulations exhibited shear thinning behaviour, indicating that viscosities of formulations decreased with increasing shear rate confirming a pseudoplastic behaviour for all samples analyzed (Figure 6A) (Figure 6B). However, formulations without cow's milk showed low viscosities (Figure 6A) whereas those with cow milk had high viscosities at different shear rates (Figure 6B). Differences in viscosities were due to the fact that formulations without cow milk did not gel. These yoghurts did not gel because they lacked lactose (enough fermenting sugars) required by the lactic acid bacteria to effect fermentation.[21] The presence of cow's milk in the other formulations boosted fermentation and resulted in the production of more lactic acid. This subsequently decreased the pH of the medium and caused proteins to precipitate out near their iso-electric point, interact through hydrophobic bonds and form stable gels. Data obtained for viscosity analysis at varying shear rates were fitted to the power law model to obtain the flow behaviour (n) and consistency (k) indices of the various SPCY formulations. The flow behaviour indices for all the soy-peanut-cow milk yoghurt formulations were less than one confirming a non-Newtonian behaviour (Figure 7). Samples without cow milk generally had low consistency indices. Consistency indices increased with increasing cow milk content and decreasing peanut milk content (Figure 8).

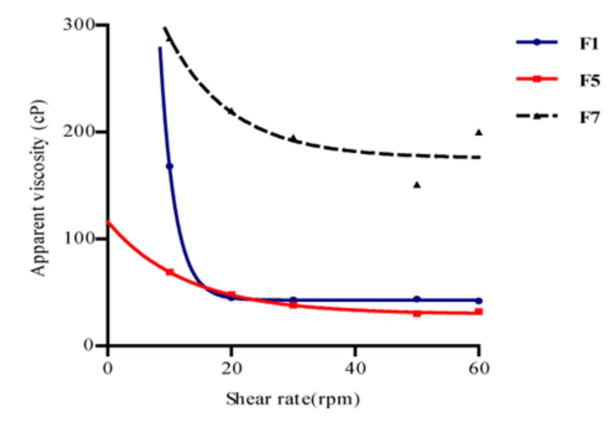

Figure 6A Graph showing viscosities of SPCY without cow's milk.

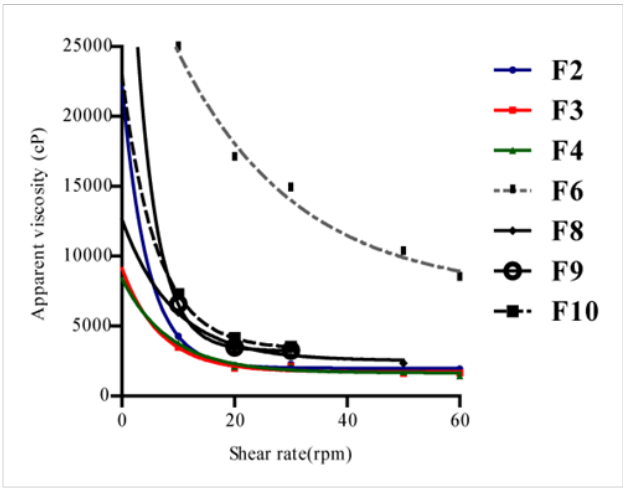

Figure 6B Graph showing viscosities of SPCY with cow's milk.

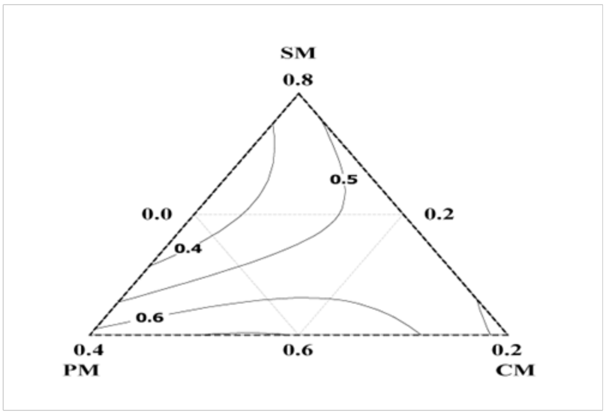

Figure 7 Mixture contour plot of flow behaviour index of SPCY.

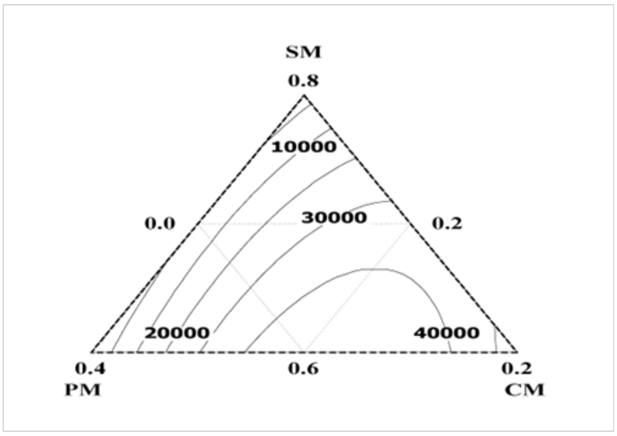

Figure 8 Mixture contour plot of consistency index of SPCY.

Water Holding Capacity (WHC) and susceptibility to syneresis of SPCY samples

WHC of a protein gel is a critical parameter in yoghurt manufacturing, since it is related to syneresis, which is due to the intrinsic instability of gels. When the protein networks of yoghurt system shows low water holing capacity, syneresis occurs and this is undesirable. Water holding capacities of yoghurt samples increased and susceptibility of yoghurt samples to syneresis decreased with increasing soy milk content (Figure 9) (Figure 10). Variations in the protein matrix of different yoghurt mixtures could lead to differences in their susceptibility to syneresis and water holding capacities.[22] Formulations without cow milk were the least susceptible to syneresis. As cow milk content increased in the samples, WHC decreased and the formulations exuded more water. WHC and STS is a property of the gel structure such that as stability of protein-protein interactions in a medium increases, a product effectively holds water and becomes less porous and thus less susceptible to syneresis. Amongst the formulations which recorded the least STS values, susceptibility to syneresis decreased as soy content increased. However an opposite trend was observed as the peanut content in the formulations increased. Formulations containing high soy protein content have a high tendency to form stable protein gels in a protein-protein mixture relative to those with high casein or peanut protein content. Soy protein gels thus have a better ability to entrap water within its three-dimensional network.[23]

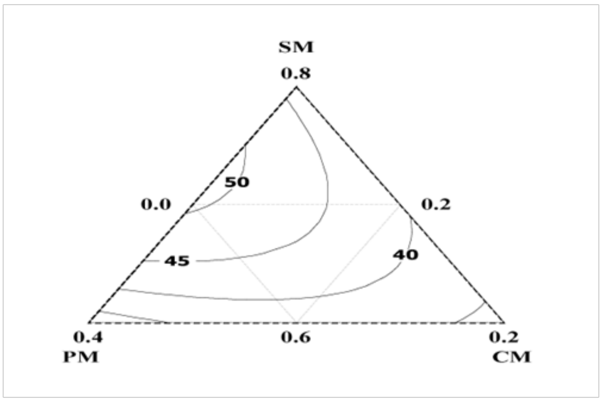

Figure 9 Mixture contour plot of WHC of SPCY formulations.

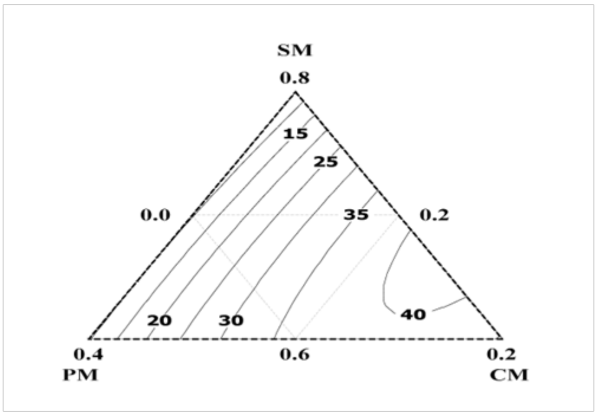

Figure 10 Mixture contour plot of STS of SPCY formulations.

Conclusion

Multiple component constrained mixture design was successfully employed to study the effect of varying ingredient on acidification and product quality characteristics of soy-peanut-cow milk yoghurt (SPCY). Ingredient variations influenced to varying extent microbial acidification and rheological characteristics of SPCY formulations. Non-volatile (titratable) acidity increased with increasing cow milk content with concomitant decreases in pH. All SPCY formulations showed similar lightness and yellowish appearance properties. Yellowness and lightness increased with increasing soymilk content.[24] The apparent viscosities of all SPCY formulations decreased with increasing shear rate confirming a pseudoplastic behaviour for all samples analyzed.[25] Formulations with cow's milk showed high viscosities at different shear rates as compared to formulations without cow's milk. The flow behaviour indices for all the soy-peanut-cow milk yoghurt formulations were less than one confirming a non-Newtonian behaviour. Samples without cow milk generally had low consistency indices. Consistency indices increased with increasing cow milk content and decreasing peanut milk content. The water holding capacities of yoghurt samples increased and yoghurt susceptibility to syneresis decreased with increasing soy milk content. Formulations without cow milk showed the least susceptible to syneresis.

Acknowledgments

None.

Conflict of interest

Author declares that there is no conflict of interest.

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