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Application of multiple component constraint mixture design for studying the effect of ingredient variations on the chemical composition and physico-chemical properties of soy-peanut-cow milk

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Abstract

Investigations were conducted employing a three-component constrained mixture design to formulate milk blends from soy milk, peanut milk and cow milk. Variations in chemical composition and physico-chemical properties of 10-soy-peanut-cow milk (SPCM) formulations were studied. Variations in soy-peanut-cow milk (SPCM) concentrations influenced to varying levels the chemical composition and physico-chemical properties of blends. SPCM formulations containing significant amounts of all three ingredients used (60-70% soy milk, 20-27% peanut milk and 7-20% cow milk) had high crude protein and fat values ranging from 2.20-2.51% and 5.00-6.35% respectively. Increasing soy concentrations caused relative increases in protein content while fat content increased with increasing peanut concentrations. SPCM formulations were high in the minerals Fe and Mn relative to cow milk which was high in Ca and Zn content. Trends in pH were contrary to titratable acidity and increased with increasing soy milk content but decreasing cow milk content. SPCM formulations demonstrated acceptable non-Newtonian behaviour and consistency indices.

Introduction

Milk is an oil-in-water emulsion containing fat droplets and protein aggregates (Cruz *et al*., 2009). Milk-like beverages manufactured from legumes such as soya beans and peanut have been noted as potential nutritional substitutes to cow milk (Beasley *et al*., 2003; Isanga and Zhang, 2009). Soy protein has been noted as an advantage over animal protein as it does not raise serum cholesterol values (Fukushima, 2001) and hence, is useful for people suffering from cardiovascular disorders (De Kleijn *et al*., 2002). Soybean milk also has a number of phytochemicals found to be effective in fighting osteoporosis (Anderson and Garner, 1997), obesity, cancer (Messina, 1999) and postmenopausal problems (Albertazzi *et al*., 1998). Soy milk is used in cases of lactose intolerance. Peanut milk also has nutritional benefits because of its richness in protein, minerals and essential fatty acids such as linoleic and oleic acids which are considered to be highly valuable in human nutrition (Isanga and Zhang, 2009). In some developing countries where dairy and dairy products are usually priced too high for low income earners and are also invariably produced in insufficient quantities, milk products from a vegetable source is an excellent alternative to help remedy the problem of protein deficiency.

Functional properties of food proteins are essential factors to consider in the formulation of new food products (Yu *et al*., 2007). Proteins impart desirable physicochemical and rheological properties like water and oil holding capacities, viscosity, emulsification, gelation, foam formation and whipping capacity to food systems (Moure *et al*., 2006; Yu *et al*., 2007). The presence of soy proteins, peanut proteins and casein in food systems and their interactions can influence physicochemical and rheological properties of such food products.

Soy-peanut-cow milk is a composite milk product formulated 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 (Cornell, 1983). 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%) (Leardi, 2009). Components cannot be varied independently because by varying the percentage of one component, percentages of the

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other components change (Leardi, 2009). The essence of combining milk from two vegetable sources is guided by the fact that no single vegetable milk can adequately resemble milk from a dairy source in its nutritional and physico-chemical attributes. Thus, the objective of the study was to apply multiple component constraint mixture design in combining milk from three different sources and investigate the effect of ingredient variations on the chemical composition and physico-chemical properties of the milk blends.

Materials and Methods

Materials

Red-skinned peanut seeds (Chinese variety) and soya bean seeds (Jenguma variety) were purchased from a registered seed grower in Tamale, Northern region of Ghana. Care was taken to ensure that good quality and mould-free seeds were selected. Cow milk used for the study was obtained from Amrahia Dairy Farms, Amrahia, Ghana.

Milk preparation

Peanut milk and soy milk were prepared by modifying the method reported by Aidoo *et al*. (2010). Sorted peanut seeds were blanched by submerging in boiling water for 10 minutes to inactivate the enzyme lypoxygenase known for its ability to cause oxidation which leads to the production of beany flavour. The seeds were then de-skinned, weighed and soaked in 2% $NaHCO_3$ for 18 hours. To remove residual $NaHCO_3$, the peanut kernels were washed with hot water (70 °C). Soy beans were also steeped in boiling water (100 °C) for about 10 minutes, dehulled, weighed and then steeped in water for 16hrs, and then in 2% $NaHCO_3$ for 2 hours. Soaking in $NaHCO_3$ was to soften the seeds and also reduce the beany flavour. 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 [oilseed (g): water (ml)] and then milled to obtain the slurry (Isanga and Zhang, 2009). The slurry was filtered to obtain a smooth, fine, homogenized milk. Cow milk was added to the prepared soy milk and peanut milk in proportions determined by a mixture design (Table 1) to obtain the soy-peanut-cow milk blend.

Experimental design

Ten milk formulations were processed 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 (Cornell, 1983). Using design of experiments software, Minitab version 14, a mixture design (centroid design) was used to obtain 10 design points from three components. The lower limit (soy milk - 0.6; peanut milk - 0.2; cow milk - 0.0) and upper bound constraints (soy milk - 0.8; peanut milk - 0.4; cow milk - 0.2) for each mixture component were used to generate the design (Figure 1).

Table 1. Design matrix for ingredient formulations of SPCM

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

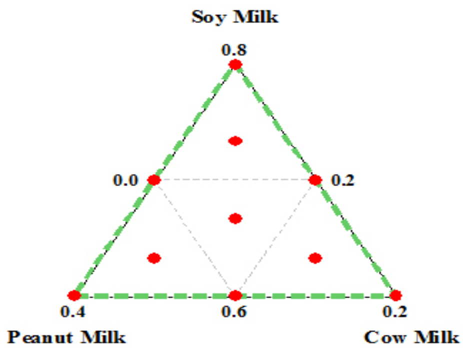

Figure 1. constrained simplex design used in formulating ingredients for vegetable milk yoghurt

Analytical methods

Proximate composition

Proximate analysis was done on the ten different formulations of soya-peanut-cow milk. Moisture, total nitrogen and ash were determined according to AOAC methods 925.09, 920.105 and 923.03 respectively (AOAC, 1990). Protein was calculated from total nitrogen using the conversion factor 6.25 for the vegetable milk. Fat content was determined by the Gerber method (AOAC, 1990). Carbohydrate was determined by difference. The calorific values were calculated using the expression:

$$EV\ (Kj/100g) = [(\%AC \times 17) + (\%P \times 17) + (\%F \times 37)]$$

where: EV = Energy value of food; %AC = Percentage available carbohydrates; %P = Percentage protein; %F = Percentage fat (Isanga and Zhang, 2009).

Mineral determination

The ash from proximate analysis was dissolved in 10 ml of 10% (v/v) nitric acid and 10% (v/v) hydrochloric acid and made up to volume with distilled water in a 25 ml volumetric flask (Isanga and Zhang, 2009). Calcium, magnesium, copper, manganese, zinc and iron in the prepared samples were determined by atomic absorption spectrophotometry (Perkin Elmer Analyst 400. Tokyo, Japan).

Titratable acidity and pH

Titratable acidity was determined using AOAC method 947.05 (AOAC, 1990) 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).

Apparent viscosity

The apparent viscosity of the 10 soy-peanut-cow milk formulations were measured at 10°C using a Brook-field viscometer (Brook-field model LVDVI, AE42086, Springfield, MA, USA). The flow curves of the milk were obtained by varying the shear rate from 10 to 60 s^{-1} and the corresponding viscosity values measured (Isanga and Zhang, 2009).

Statistical analysis

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

Results and Discussion

Proximate composition of soy-peanut-cow milk (SPCM)

The proximate composition of the 10 soy-peanut-cow milk formulations were determined. The solids content of the soy-peanut-cow milk formulations ranged from 8.47 to 9.68% and decreased with increasing soymilk content (Figure 2). SPCM formulations did not show any great difference in solids content when compared with other soy milk. Fávaro-Trindade *et al*. (2001) and Gatade *et al*. (2009) observed high moisture content above 90% in soy milk. However Isanga and Zhang (2009) reported lower moisture content (87.15%) peanut milk. Since the formulations used in this study generally had high amounts of soy milk than peanut milk, moisture content of samples were high and comparable to that of soy milk (Fávaro-Trindade *et al*., 2001; Gatade *et al*., 2009). Formulations without cow milk had lower

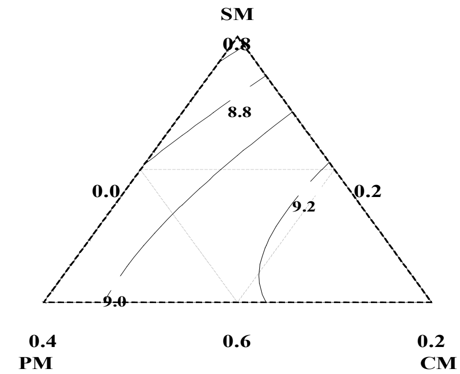

Figure 2. contour plot of total solids content of SPCM formulations

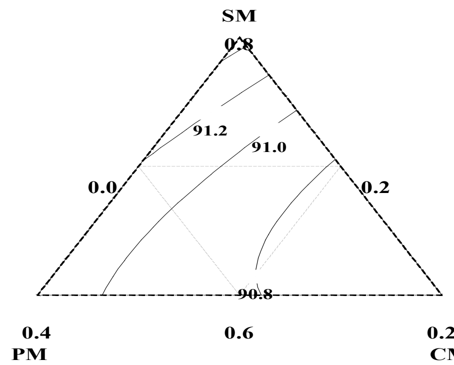

Figure 3. contour plot of moisture content of SPCM formulations

solids content 8.47 to 8.94% relative to formulations that had cow milk. Cow milk generally has been observed to have higher total solids content than other aqueous extracts of oil seeds (Schaffner and Beuchat, 1986; Vargas *et al*., 2008). Hence it is expected that as the proportion of cow milk increases in a mixture relative to vegetable milk, moisture content correspondingly decreases. The moisture content of formulations increased with increasing soy milk content (Figure 3). Since the vegetable milk was an aqueous plant seed extract it contained more moisture as compared to cow milk. The solids content of SPCM formulations with different proportions of soy milk and peanut milk were different but formulations without cow milk did not differ significantly ($p > 0.05$). Among formulations that had cow milk ranging from 3-10%, there were no significant differences ($p > 0.05$) between their solids content.

Crude protein content of the formulations ranged from 1.77% to 2.59% and the values were significantly different for all samples which contained varied proportions of soy milk, peanut milk and cow milk. In this study formulations which contained significant amounts of all three components: 60 to 70% soy

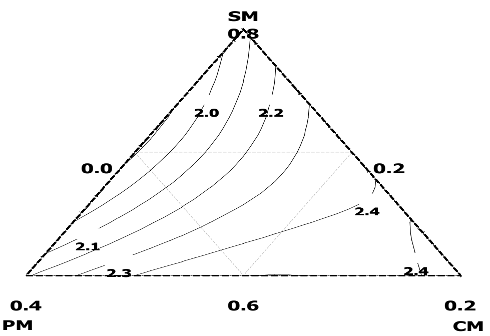

Figure 4. contour plot of protein content of SPCM formulations

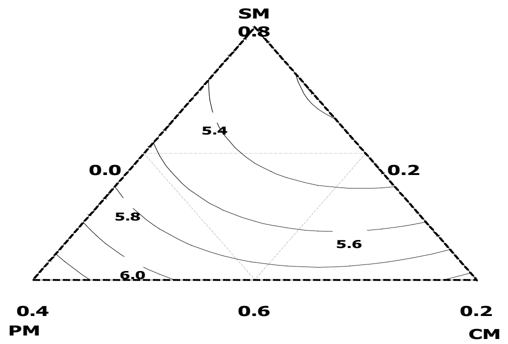

Figure 5. contour plot of fat content of SPCM formulations

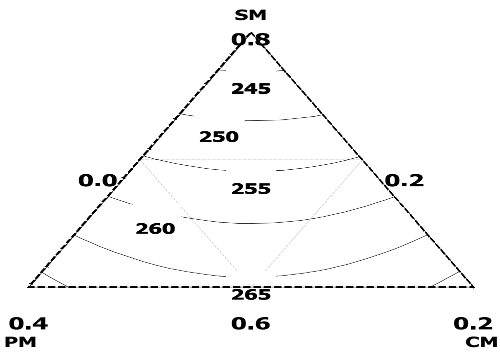

Figure 6. contour plot of energy value of SPCM formulations

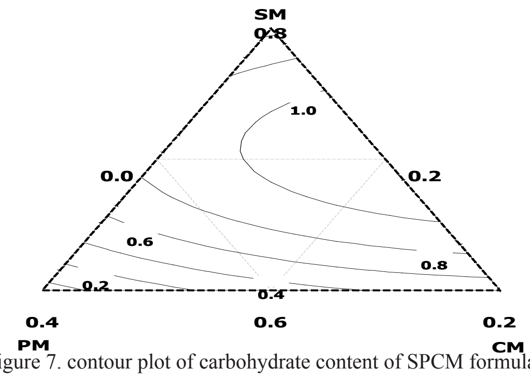

Figure 7. contour plot of carbohydrate content of SPCM formulations

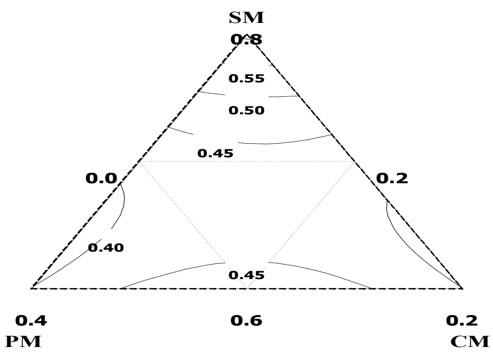

Figure 8. contour plot of ash content of SPCM formulations

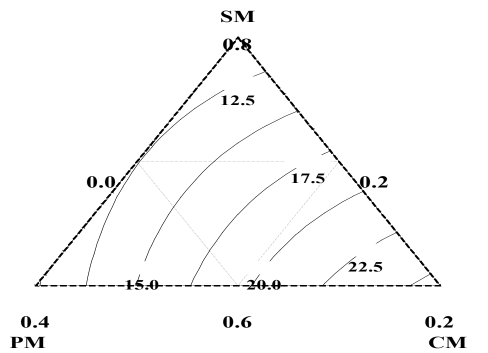

Figure 9. Mixture contour plot of Ca content of SPCM formulations

milk; 20 to 27% peanut milk and 7 to 20% cow milk generally had high crude protein values ranging from 2.20 to 2.51%. The crude protein content of the samples increased when all components of the mixture were present in high amounts (Figure 4). Crude protein content of soy milk (4.8%) and peanut milk (3.71%) were generally higher than cow milk (2.84%) primarily because these oil seeds are the most abundant sources of proteins (Gatade *et al*., 2009; Isanga and Zhang, 2009).

The fat content of soy-peanut-cow milk samples ranged from 5.00 to 6.35%. The fat content increased with increasing peanut content in the formulations (Figure 5). Values obtained for fat content were lower than the value of 8% reported by Isanga and Zhang (2009) for peanut milk prepared from roasted peanut seeds. Since the fat content of peanut is greater than the other components of the mixture (soy beans and cow milk), an increase in peanut content expectedly increased the fat content of the formulations. The energy value of formulations ranged from 238.45 to 274.70 KJ/100g and increased with increasing peanut milk but decreasing soy milk content (Figure 6). The high fat content of peanut milk might have contributed to the high energy values recorded for the high peanut containing formulations.

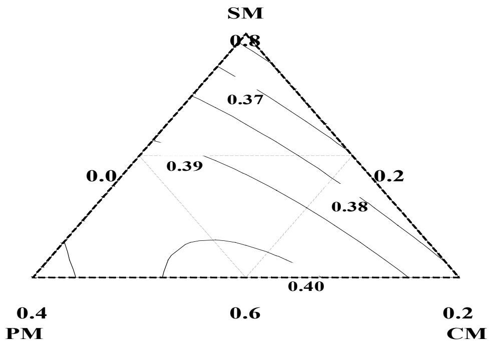

Figure 10: Mixture contour plot of Zn content of SPCM formulations

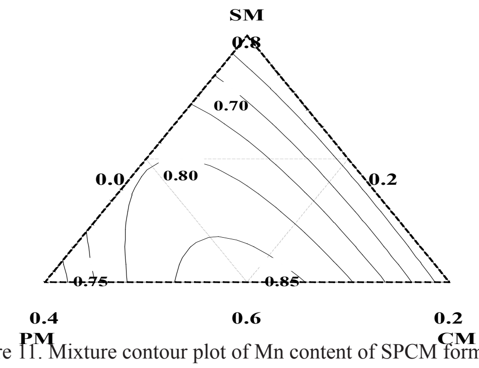

Figure 11. Mixture contour plot of Mn content of SPCM formulations

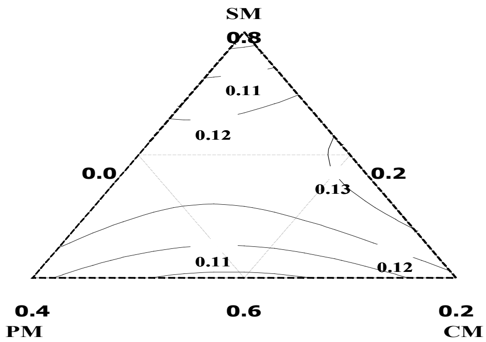

Figure 12. contour plot of Fe content of SPCM formulations

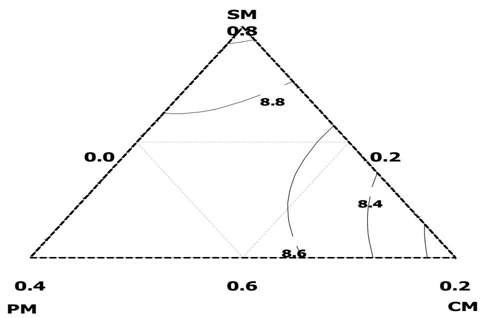

Figure 13. contour plot of pH of SPCM formulations

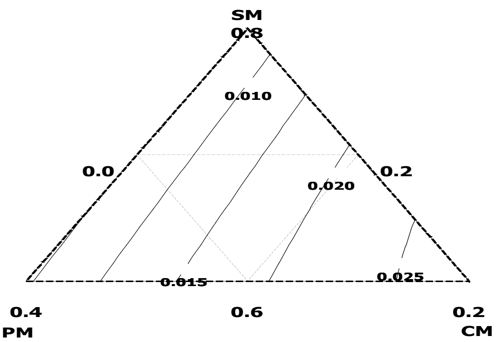

Figure 14. contour plot of titratable acidity of SPCM formulations

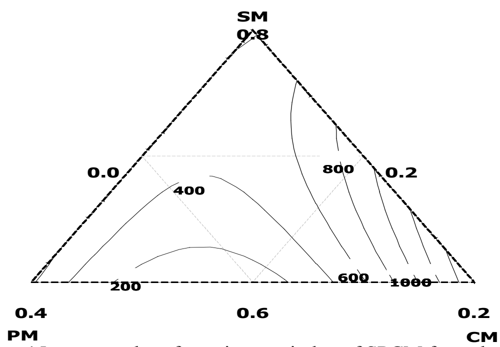

Figure 15.contour plot of consistency index of SPCM formulations

The carbohydrate content in the formulations ranged between 0.10 to 2.09%. The carbohydrate content of samples increased as proportions of soy milk and cow milk increased in samples (Figure 7). The carbohydrate content of aqueous extracts of oil seeds is usually low due to their high protein and fat content. However, soy milk generally has higher carbohydrate content than peanut milk. Values between 1.5 to 2.0% for soy milk (Gatade *et al*., 2009) and 0.84 to 0.95% for peanut milk (Schaffner and Beuchat, 1986; Isanga and Zhang, 2009) have been reported.

The ash content of a food material is the inorganic residue remaining after the organic material has been burnt off. The importance of ash content is that it gives an idea of the amount of mineral elements present in the food sample. The ash content of SPCM mixtures ranged from 0.38 to 0.61%, and the values did not differ significantly ($p > 0.05$) between all the 10 formulations. However increasing soymilk and cow milk in formulations increased ash content (Figure 8).

Mineral composition of soy-peanut-cow milk samples

The formulations generally had high Fe and Mn

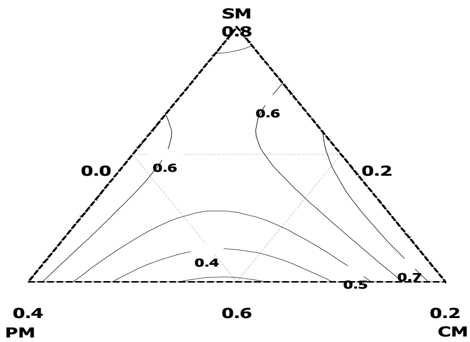

Figure 16.contour plot of flow behaviour indices of SPCM formulations

content compared to the control, cow milk, which had high Ca and Zn content. As expected, formulations without cow milk were low in Ca content. Ca and Zn content increased in formulations as cow milk content increased (Figures 9 and 10). Manganese increased with increasing peanut milk content and decreasing soy milk content (Figure 11). Increasing soymilk content also increased Fe content of formulations (Figure 12).

Titratable acidity and pH of soy-peanut-cow milk (SPCM)

Titratable acidity of a food system is indicative of the total acid concentration within a food and it is a better predictor of an acid's impact on flavour than pH (Nielsen, 1998). The ability of microorganisms to grow in a specific food is dependent on the hydroxonium ion concentration of the food system (Nielsen, 1998). pH values ranged from 8.06 to 9.08 and titratable acidity was between 0.005 and 0.027%. The results of the pH measurements were contrary to trends observed for titratable acidity measurements. As the acidity increased, the pH decreased (Figures 13 and 14). The pH of soy milk ranges between 6.5 to 6.8 (Pinthong *et al*., 1980; Buono *et al*., 1990; Park *et al*., 2010). However the pH of SPCM formulations observed in this study were far higher than those reported in literature. This could be due to the fact that the seeds were soaked in a solution of $NaHCO_3$ prior to milk extraction and the $NaHCO_3$ solution might have increased the pH of the aqueous extracts from these seeds. The pH values of SPCM formulations differed significantly ($p < 0.05$). pH values generally increased with increasing soy milk content and decreasing cow milk content (Figure 13). Formulations with either no or low amount of cow milk had high pH values in the range of 8.7 to 8.8 and low total acid content ranging from 0.005 to 0.016%. Formulations with cow milk content ranging from 10 to 20% recorded low pH and high titratable acidity values. The titratable acidity of SPCM formulations without cow milk did not differ significantly ($p > 0.05$).

Rheological characterization of SPCM

Viscosity is an important property of fluid foods that affects mouth feel and consistency. The flow behavior of fluid foods affects the efficacy of unit operations in the food industry, notably pumping, extrusion, filling, drying and evaporation. Viscosity measurements are therefore routinely performed in the food industry and research laboratories, primarily as part of quality control or product development (Yu *et al*., 2007).

Data obtained from apparent viscosity determination of the formulations at varying shear rates were fitted to the power law model to obtain the consistency and flow behaviour indices of SPCM formulations. The consistency index of SPCM formulations increased with increasing cow milk content and decreased as peanut milk increased in samples (Figure 15). SPCM formulations without cow milk generally had low consistency indices. Since the total solid content of cow milk is higher than that of vegetable milk, an increase in cow milk content should increase the consistency/viscosity of the milk products. It had been reported that higher total solids content in milk usually increases the viscosity and consistency of the end product (Tamime and Robinson, 1999). Yoghurt manufacturers in an attempt to develop yoghurt products with high viscosities and better consistencies would usually add powdered milk to the raw material to increase the total solids content and produce yoghurts with improved consistencies and viscosities. The flow behaviour indices for all the soy-peanut-cow milk formulations showed them to have non-Newtonian flow behaviour. Unlike cow milk that has been established to be Newtonian, the flow characteristics of the formulations were pseudoplastic, with the apparent viscosities decreasing as the shear rates were increased. The flow behaviour of the mixtures gradually moved towards Newtonian flow (with the flow behavior indices approaching unity) as cow milk in the formulation increased (Figure 16).

Conclusion

Variations in soy-peanut-cow milk (SPCM) concentrations influenced the chemical composition and physico-chemical properties of blends. SPCM formulations containing significant amounts of all three ingredients used (60 – 70% soy milk, 20 – 27% peanut milk and 7 – 20% cow milk) had high crude protein and fat values ranging from 2.20 to 2.51% and 5.00 to 6.35% respectively. Increasing soy concentrations caused relative increases in protein content while fat content increased with increasing peanut concentrations. SPCM were high in the minerals Fe and Mn relative to the control (cow milk) which was also relatively higher in Ca and Zn content. Trends in pH measurements were contrary to those observed for titratable acidity and these generally increased with increasing soy milk content and decreasing cow milk content. The flow behaviour indices for all the soy-peanut-cow milk formulations were less than one confirming a non-Newtonian behaviour. The apparent viscosities of the samples decreased with increasing shear rate also confirming a pseudoplastic behaviour and samples without cow milk generally had low consistency indices. Soy-peanut-cow milk can be employed in the industrial manufacture of dairy products such as milk beverages and yoghurt with improved nutritional value and acceptable souring and acidification production. Strong interactions between soy proteins, peanut proteins and casein could generate a stable protein gel with reduced syneresis as compared to cow milk only products.

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References

Aidoo, H., Sakyi-Dawson, E., Tano-Debrah, K. and Saalia, F.K. 2010. Development and characterization of dehydrated peanut–cowpea milk powder for use as a dairy milk substitute in chocolate manufacture. Food Research International 43: 79-85.

Albertazzi, P., Pansini, F., Bonaccorsi, G., Zanotti, L., Forini, E. 1998. The effect of dietary soy supplementation on hot flushes. Obstetrics and Gynecology 91: 6–11.

Anderson, J.B. and Garner, S.C. 1997. The effect of phytoesterogens on bones. Nutrition Research 17: 1617–1632.

AOAC. 1990. Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Washington, D.C.

Beasley, S., Tuorila, H. and Saris, P.E. 2003. Fermented soymilk with a monoculture of *Lactococcus lactis*. International Journal of Food Microbiology 81: 159–162.

Buono, M.A., Setser, C., Erickson, L.E. and Fung, D.C. 1990. Soymilk yoghurt: sensory evaluation and chemical measurement. Journal of Food Science 55: 528-531.

Cochran, W.G., Cox, G.M. 1957. Experimental design. 2nd edn. John Wiley and Sons, New York, USA.

Cornell, J.A. 1983. How to run mixture experiments for product quality. American Society for quality control, Milwaukee, WI.

Cruz, N.S., Capellas, M., Jaramillo, D.P., Trujillo, A.J, Guamis, B. and Ferragut, V. 2009. Soymilk treated by ultra high-pressure homogenization: acid coagulation properties and characteristics of a soy-yoghurt product. Food Hydrocolloids 23: 490 – 496.

De Kleijn, M.J., Vander-Schouw, Y.T., Wilson, P.W., Grobbee, D.E. and Jacques, P.F. 2002. Dietary intake of phytoestogens is associated with a favourable metabolic cardiovascular risk profile in postmenopausal U.S. women: the Framingham study. Journal of Nutrition 132: 276 – 282.

Fávaro-Trindade, C.S., Terzi, S.C., Trugo, L.C., Della-Modesta, R.C. and Couri, S. 2001. Development and sensory evaluation of soy milk based yoghurt. Organo Oficial de la Sociedad Latinoamericana de Nutrición 51: 76-83.

Fennema, O.R. 1997. Food Chemistry. 3rd edn. Marcel Dekker, Inc. 270 Madison Avenue, New York, USA..

Fukushima, D. 2001. Recent progress in research and technology on soybeans. Food Science and Technology Research 7: 8–16.

Gatade, A.A., Ranveer, R.C. and Sahoo, A.K. 2009. Physico-chemical and sensorial characteristics of chocolate prepared from soymilk. Advance Journal of Food Science and Technology 1: 1-5.

Isanga, J. and Zhang, G. 2009. Production and evaluation of some physicochemical parameters of peanut milk yoghurt. Food Science and Technology 6:1132-2238.

Leardi, R. 2009. Experimental design in chemistry: a tutorial. Analytica Chimica Acta 652:161 – 172.

Messina, M.J. 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. American Journal of Clinical Nutrition 70: 439 – 450.

Moure, A., Sineiro, J., Dominguez, H. and Parajo, J.C. 2006. Functionality of oilseed protein products: A review. Food Research International 39: 945 – 963.

Nielsen, S.S. 1998. Food analysis. 2nd edn. Aspen Publishers, Inc. New York, U.S.A.

Park, D.J., Oh, S., Hyunkgku, K., Mok, C., Kim, S.H. and Imm, J. 2005. Characteristics of yoghurt-like products prepared from the combination of skim milk and soymilk containing saccharified-rice solution. International Journal of Food Science and Nutrition 56: 23 – 34.

Pearson, D. 1976. The chemical analysis of foods. 7th edn. Churchill Livingstone Edinburgh London.

Pinthong, R., Macrae, R. and Rothwell, J. 1980. The development of a soya-based yoghurt I. Acid production by lactic acid bacteria. Journal of Food Technology 15: 647 – 652.

Schaffer, D.W. and Beuchat, L.R. 1986. Fermentation of aqueous plant seed extracts by lactic acid bacteria. Applied and Environmental Microbiology 51: 1072 – 1076.

Vagas, M., Chafer, M., Albors, A., Chiralt, A. and Gonsalez-Martinez, C. 2008. Physicochemical and sensory characteristics of yoghurt produced from mixtures of cows' and goats' milk. International Dairy Journal 18: 1146 – 1152.

Yu, J., Ahmedna, M. and Goktepe, I. 2007. Peanut protein concentrate: production and functional properties as affected by processing. Food Chemistry 103: 121 – 129.