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ORIGINAL ARTICLE

Stability and quality of herb (*Pueraria Tuberosa*)-milk model system

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Abstract The medicinal benefits of herbs could be conveyed via certain foods as carriers. Milk is one of the important carrier which has been effectively used to deliver phytochemicals presents in herbs (mainly polyphenols) for targeted health benefits in the traditional Indian system of medical science. The present study was conducted to evaluate the effect of herb components (*Pueraria tuberosa*) on properties of *Pueraria tuberosa*-milk model system. The herb was added into cow milk on the basis of sensory evaluation (0.4 %) by using 9-point hedonic scale. The physical and chemical changes were evaluated after various processing treatments viz. pasteurization (72 °C, 15 s), sterilization (121 °C, 15 min), separation etc. These changes were determined using viscosity, hydroxy methyl furfural (HMF) value, ethanol stability, colour characteristics and trolox equivalent antioxidant capacity (TEAC). It was observed that addition of *Pueraria tuberosa* to milk resulted in decreased HMF content, ethanol stability and lightness whereas antioxidant activity, redness and yellowness increased as compared to control. It can be concluded that addition of *Pueraria tuberosa* to milk at 0.4 % concentration altered the functional properties of milk and *Pueraira tuberosa* could be suitable for preparation of low heat treated functional dairy food products.

Keywords Milk · *Pueraria tuberosa* · 9-point hedonic scale · pH · Colour characteristics · Ethanol stability · TEAC · Polyphenol content

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Introduction

Herbal nutraceuticals are commonly used by people who seek alternative health care for prevention and treatment of disease. Therefore in the recent past there has been rapid growth in demand for herbal medicines in food preparations. According to WHO, The estimated world market of herbal products were 62 billion US (United States) dollars which is projected to grow to US $ 5 trillion by 2050 (Singh 2006). *Pueraria tuberosa* (family-*Fabaceae*) is one such important and potential medical plant in traditional and folklore system, commonly known as *Pueraria tuberosa* & Indian Kudzu. Its tuber is widely used as active component in various formulations of Indian system of medicine (*Ayurveda*). It has been used as an aphrodisiac, cardiotonic, diuretic, anti-carcinogen, anti-diabetes, hypolipidemic (Tanwar et al. 2008), an antioxidant under in vitro and in vivo condition (Verma et al. 2009).

Milk is considered one of the most effective carrier medium for delivering bioactives or phytochemicals in the traditional Indian system of medical science i.e. *Ayurveda*. There are evidences to suggest that addition of polyphenols (mainly phenol) into milk from certain herb increased antioxidative (free radical scavenging) stability, heat stability (O'Connell and Fox 1999) but reduced non-enzymatic browning (Gerry and Theodore 2006), Rennet Clotting Time (O'Connell and Fox 1999) where as no change could be noted in ethanol stability (O'Connell and Fox 1998). Currently, polyphenol-rich extracts from green tea (catechins) are being incorporated into fruit-flavoured milk drinks, as well as into products such as chewing gum and sweet paste biscuits. The production of soy-whey protein based yoghurt using an isoflavone-enriched soy protein preparation (2.5 mg isoflavone/g) has been patented and it is claimed that in addition to providing a source of dietary isoflavones, the resulting yoghurt had superior organoleptic properties (Crank and Kerr 1999). The addition of polyphenol-rich

wine extract to milk and yoghurt products, as a nutritional additive, has also been patented (Howard et al. 2000). The antioxidative effect of herbal *sandesh* decreased in the order: turmeric > curry leaf > aonla > spinach > coriander leaf. (Bandyopadhyay et al. 2007). These modifications in properties of the milk system are of great technological significance as they may significantly alter processing during parameters for the manufacture of the products significantly.

The isoflavonoids of *pueraria tuberosa* could interact with milk proteins viz., bovine serum albumin (Liu 2009), casein micelle (Juqun 2008) and β-lactogobulin as have been reported in case of certain food and drug preparations containing soya isoflavonoids. Such data on the stability of bioactive molecules of *Pueraria tuberosa* in model dairy food systems and their interaction with major milk constituents which is necessary to establish efficacy of their use as nutraceutical in dairy foods are not available in the literature. Hence, the present study was conducted to investigate the effect of herb (*Pueraria tuberosa*) in an herb-milk model system.

Materials and method

Materials

The freeze dried hot water extract of herb (*Pueraria tuberosa*) was procured from National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh, India. N,N'-Methylene-bis-acrylamide; N,N,N',N'-Tetramethylethylenediamine; Sodium lauryl sulphate; Tris(hydroxymethyl) aminomethane; 2-Mercaptoethanol; Acrylamide; Hydroxy methyl furfural; Ammonium persulphate were purchased from HIMEDIA, Mumbai. All the reagents used were of analytical grade.

Preparation of *Pueraria tuberosa*-milk model

Fresh cow milk was collected from Cattle Yard, National Dairy Research Institute, Karnal, India and warmed at 40–45 °C. The warm milk was filtered using muslin cloth to remove any foreign materials. Lyophilized extract of *Pueraria tuberosa* was crushed using pestle and mortar with small quantity of milk and then the mixture was added to bulk milk. The milk was then evaluated for different processing treatments e.g. pasteurization, sterilization etc. for further analysis.

Sensory evaluation

The milk-herb mixture prepared was evaluated for sensory characteristics by a panel of 5 trained judges selected from the institute. Four levels of herb (0.2, 0.3, 0.4 and 0.5 %) were tried and the best one was selected through 9-point hedonic scale score card, ranging from 'like extremely=9' to 'neither like nor dislike=5' to 'dislike extremely=1'. Panel members were directed to judge for flavour, body and texture, colour and appearance, mouth feel and overall acceptability and requested to indicate their score on score card for herbal milk.

Methods

Viscosity

The viscosity of milk and herbal milk was measured at 20°C using coaxial cylinder rotational Viscometer visco star plus (FUNGILAB, S. A.) with digital display (fitted with probe TL5 to the spindle) at shear rate 16.8 s^{-1} (60 rpm).

Ethanol stability

Two ml of ethanol solution varying from 0 to 100 %, v/v, and ethanol (in increments of 2.5 % ethanol) were added to two ml (in petridishes) control and experimental milk samples. The mixtures were rotated and subjectively examined for any visible sign of protein flocculation. The minimum concentration of ethanol necessary to destabilise milk proteins was recorded as ethanol stability (Davies and White 1958).

Colour characteristics

The colour of the control and experimental milk samples were measured using a Colourflex (Hunterlab, Reston, Verginia, USA) along with the universal software (Version \$.10). The light source was dual beam xenon flash lamp. Data was received through the software in terms of L* (Lightness) ranging from Zero (black) to 100 (white), a* (Redness) ranging from +60 (red) to −60 (green) and b* (Yellowness) varying from +60 (yellow) to −60 (blue) of the international colour system

Hydroxy methyl furfural (HMF) value

The quantitative method presented by Keeney and Bassette (1959) for quantifying HMF by spectroscopic method was used to assess the extent of browning in milk. Ten ml of sample was pipetted into a 50 ml test tube and to this 5 ml of 0.3 N oxalic acid was added and mixed thoroughly. The tubes were covered with inverted 20 ml glass beaker and placed in a boiling water bath for 1 h and cooled to room temperature. Thereafter, 5 ml of 40 % TCA was added, the contents mixed thoroughly and subsequently filtered through a Whatman filter paper No.42. Four ml of filtrate was pipetted into a test tube, followed by addition of 1 ml of 0.05 M TBA. The test tube was placed in water bath maintained at 40°C for 40 min and subsequently cooled to room temperature. The solution was then subjected to measurement of absorbance at 443 nm in a spectrophotometer. A

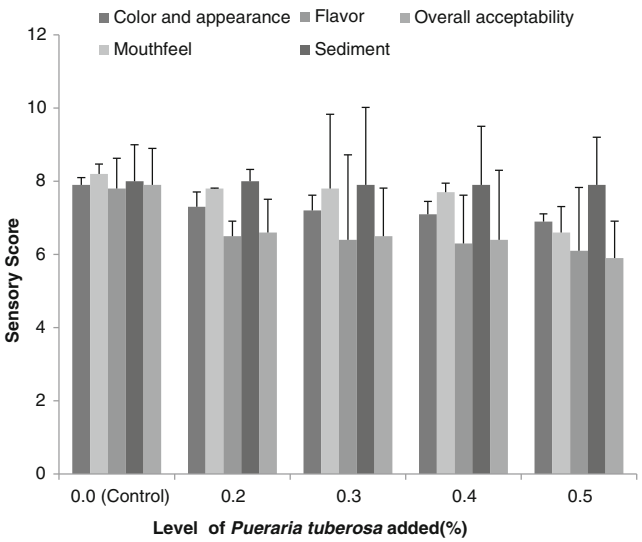

Fig. 1 Effect of addition of different levels of *Pueraria tuberosa* added (%) on sensory quality of pasteurized milk. Data are presented as means±SEM (n=5). Means in each row with different superscripts (*a*, *b*, *c*) were significantly different (P<0.05) from each other. Statistical comparisons were made between samples using ANOVA single factor

blank was simultaneously prepared using water instead of herbal milk. The absorbance of the sample was measured against the blank. HMF was calculated from the absorbance which is the total of free and potential HMF formed from browning intermediates. The standard curve was prepared by plotting concentration (um/l) of HMF v/s absorbance at 443 nm.

Determination of trolox equivalent antioxidant capacity (TEAC)

Free radical scavenging activity of samples was determined by ABTS method (Re et al. 1999). ABTS (3 ml) working solution made with PBS (pH 7.4) was added to cuvette (3 ml capacity) and absorbance adjusted to 0.7±0.02 against the buffer. Four (μl/ml) of sample was added to ABTS working solution as well as to the blank. The contents were mixed for 5 s and change in absorbance at 734 nm was recorded over 10 min using SPECORD-200 double beam spectrophotometer (Analytical zena, Germany). The standard curve was prepared by plotting concentration (μM) of Trolox v/s % inhibition. The % inhibition of absorbance of sample and trolox equivalent was determined from using following equation and standard curve respectively.

$$\%\ \text{inhibition} = \left[\left(A_{734nm}\text{Control}–A_{734nm}\text{Sample}\right)\Big/A_{734nm}\text{Control}\right] \times 100$$

Statistical analysis

All statistical analyses were performed using SYSTAT 6.0.1 software. Results are presented in means±standard error of mean (SEM), and statistical significance was set at p<0.05. ANOVA single factor was used to determine the main effects of treatments. All experiments were carried out in triplicate.

Results and discussion

Optimization of oraganoleptically acceptable levels of addition of *Pueraria tuberosa* extracts in milk

The acceptability of milk added with herb extract was sensory evaluated and results statistically analyzed by using one factor ANOVA to standardize the level of herb in cow milk (Fig 1). As the concentration of *Pueraria tuberosa* extract was increased from 0.2 to 0.5 %, a significant drop (p<0.05) in all the sensory attributes except sedimentation was observed. Milk with 0.2 % extract showed slightly astringent flavour (puckering, rough or drying mouth feel) and greenish colour. Good quality cow milk should be yellowish in colour with pleasantly sweet flavour without any sediment. *Pueraria tuberosa* is rich in isoflavonoids (Puerarin, daidzein, genistein

Table 1 Effect of addition of *Pueraria tuberosa* (0.4 %) on net increases in HMF (heated milk—raw milk) value after treatment

Treatments	Net increases in HMF value after treatment (μM/liter)[#]	
	Control	*Pueraria tuberosa*+milk
(Pasteurized milk-Raw milk)	1.2 ± 0.67^{aA}	1.0 ± 0.02^{bA}
(Boiled—Raw milk)	3.5 ± 0.12^{aB}	2.4 ± 0.01^{bB}

[#] Mean±SEM, n=3, different superscripts (a, b) in each row and in each column (A, B) differ significantly within groups (P<0.05). Statistical comparisons were made between samples using ANOVA single factor. *HMF* Hydroxyl Methyl Furfural

Table 2 Effect of addition of *Pueraria tuberosa* @0.4 % on color of milk

Treatments	L*		a*		b*	
	Control	Milk+*Pueraria tuberosa*	Control	Milk+*Pueraria tuberosa*	Control	Milk+*Pueraria tuberosa*
Raw milk	82.1 ± 0.08^{aA}	78.4 ± 0.41^{bA}	-1.8 ± 0.12^{aA}	-0.85 ± 0.018^{bA}	8.7 ± 0.19^{aA}	10.6 ± 0.03^{bA}
Pasteurized milk	82.6 ± 0.26^{aB}	79.2 ± 0.36^{bB}	-1.7 ± 0.030^{aA}	-0.95 ± 0.014^{bA}	8.6 ± 0.04^{aA}	10.6 ± 0.03^{bA}
Sterilized milk	83.6 ± 0.03^{aC}	80.6 ± 0.085^{bC}	-1.8 ± 0.5^{aA}	-0.84 ± 0.08^{bA}	8.8 ± 0.04^{aB}	11.3 ± 0.00^{bB}

Data are presented as means±SEM (n=3). Means with the different superscripts (a, b) in a row and in a column (A, B) are significantly different (P<0.05) from each other. Statistical comparisons were made between samples using ANOVA single factor

etc.) and polyphenols (anthocyanin). This perceived astringency in herb added milk could be therefore primarily attributed to the interaction of polyphenol—salivary protein (Peleg et al. 2004) while colour change might have been due to the presence of colouring pigments like anthocyanin in *Pueraria tuberosa* extract. No significant difference was observed in all the sensory attributes among the milk samples added with 0.2, 0.3 and 0.4 % *Pueraria tuberosa*. It indicated that there is possibility of incorporating herb extract up to 0.4 % in milk. Hence, for further investigations, freeze dried hot water extract of *Pueraria tuberosa* at 0.4 % was selected.

Hydroxyl methyl furfural (HMF) value

Increase in HMF value of *Pueraria tuberosa* added milk may be due to the presence of colouring present in the herb extract. In order to nullify the effect of these colouring compounds on HMF reading, the HMF values of *Pueraria tuberosa* added milk was considered as blank over heat treated samples. As can be seen from Table 1, *Pueraria tuberosa* extract was more effective in retarding browning (indicated by net increase in HMF content) in heat treated milk-extract mixture (pasteurized and boiled) as compared to untreated samples (raw milk—extract mix). This corroborated well the observation of Gerry et al. (2006) who reported the ability of green tea flavonoids to control maillard browning in UHT milk. Likewise, the caffeic acid was also found to reduced the browning in heated milk by nearly 10 % by blocking of the ε-amino groups of lysine in maillard reaction (Walstra and Jenness 1988). Similar reactions might have led to lesser availability of lysine molecules for maillard reaction during the present investigation.

Colour indices

The colour parameter measured with colourflex was described in terms of L* (lightness index), a* (redness index) and b* (yellowness index) values. The colour characteristics of milk and milk added with *Pueraria tuberosa* extract were studied in raw, pasteurized and sterilized milk. The L* value was significantly (p<0.05) different for all treatments given to both the control and milk added with herbal extract. In both the control and milk-herb extract mixture, lightness index increased as the heating temperature was increased (Table 2). There was significant difference (p<0.05) observed for a* value between milk and milk-herb extract mix subjected to different treatments viz. raw, pasteurization and sterilization. However, no significant difference was observed among the treatments either in case of control or samples added with herbal extract. The b* values were not significantly affected by pasteurization in case of either control samples or samples added with herb extract. Sterilization, however, affected the yellowness index significantly (P<0.05) in comparison to no

Table 3 Effect of addition of *Pueraria tuberosa* extract on antioxidant capacity of milk

Treatments (10× dilution)	Trolox equivalent/liter [#]			
	Control		*Pueraria tuberosa*+milk	
	%Inhibition	mM/liter	%Inhibition	mM/liter
Raw milk	43.30	0.857 ± 0.87^{aA}	54.35	1.1 ± 0.89^{bA}
Pasteurized milk	62.25	1.2 ± 0.56^{aB}	68.85	1.4 ± 0.98^{bB}
Sterilized milk	68.4	1.4 ± 0.23^{aC}	68.55	1.4 ± 0.09^{aB}

[#] Mean±SEM, (n=3). Different superscripts (a, b) in each row and in each column (A, B, C) means significant difference between groups (p>0.05). Statistical comparisons were made between samples using ANOVA single factor

Table 4 Influence addition of *Pueraria tuberosa* extract on ethanol stability at pH 6.71

System (Cow milk)	Ethanol Stability (%)[#]
Control	81.4±0.42[a]
With *Pueraria tuberosa*	76.0±0.26[b]

[#] Mean±SEM (n=3). Different superscripts in each column (a, b) means significant difference between groups (p>0.05). Statistical comparisons were made between samples using ANOVA single factor

treatment (raw) or pasteurization treatment given to both the control and milk-herb extract mix. The result corroborate with the study of Karaaslan et al. (2011), who found that addition of different extracts of grape varieties (1 %) into yogurt decreased L* value and increased a* value. Phenols have been associated with the colour change, particularly lightness as reported by Mondy and Gosselin (1988). Wegrzyn et al. (2008), also reported that a* values of polyphenol-milk systems were influenced by apple polyphenol concentration, storage temperature and storage duration. Addition of anthocyanins in ice-cream and other dairy products (0.3–0.5 % w/w) can impart deep red colour (Rayner 1991) but upon heating to a certain degree anthocyanin becomes paler. Addition of *Pueraria tuberosa* extract to milk resulted in significant decrease (p<0.05) in lightness while an increase in yellowness and redness, which could be mainly ascribed to the presence of anthocyanins in the herb extract.

Total antioxidant capacity (TAC) by ABTS method

It can be observed from Table 3 that milk-*Pueraria tuberosa* mix (1.101 mM/l) had significantly (p>0.05) better radical scavenging activity as compared to control milk (0.857 mM/l). The antioxidant activity was affected by heat treatment. However, significant (p<0.05) increase was observed only in case of pasteurization but not in the case of sterilization. Milk contains compounds such as urate, proteins, carotenoids and vitamins, which exhibit antioxidant activity (Calligaris et al. 2004; Chen et al. 2003). According to Gad and Salam (2010), the addition of green tea and rosemary extract to skim milk significantly increased the antioxidant activity of skim milk. They also reported that the antioxidant activity and phenol content increased on heat treatment (65 °C/30 min) of skim milk. This could be due to excess phenol released due to breaking off bonds between polyphenols and milk protein in the complexation compound or may be due to formation of radical scavenging complexation compounds formed by covalent interaction between oxidised phenolic compounds and amino acid residues of proteins (Arts et al. 2001; Ridle and Hagerman 2001; Rohn et al. 2004). Karaaslan et al. (2011) also found higher antioxidant power in grape extract fortified yogurt with higher anthocyanin content than control yogurt. Pawar et al. (2012) incorporated different herbs into ghee and they found that antioxidative activity of the herbs decreased in the order- vidarikand (*Pueraia tuberosa*)>ashwagandha>-shatavari. Modi (2009) reported that the incorporation of mango pulp enhanced the antioxidant activity of sweetened mango *dahi* by1.64 fold as compared to control sweetened *dahi*. Similarly, the antioxidant capacity of strawberry *dahi* (8 % fruit pulp) was 1.66 fold more as compared to sweetened *dahi*. They attributed the improved anti-oxidative activity of fruit pulp added *dahi* to the incorporation of carotenoids, polyphenols and anthocyanins—the natural antioxidants present in fruit pulp.

Pandey et al. (2007) evaluated the antioxidative potential of different fractions of *Pueraria tuberosa* extracts and reported that the antioxidant activity as determined by lipid peroxidation in egg yolk is due to isoflavones (Diadzein, genestein, Puerarin) present in extracts. In the present investigation, the increase in antioxidant activity of *Pueraria tuberosa* extract added milk could also be due to the incorporation of these isoflavones in milk. Increase in antioxidant activity upon pasteurization (72°C/15s) of herb extract-milk mix might be ascribed either to formation of novel antioxidant components or the interaction of protein and phenolic compounds. The reverse phenomenon was however noticed in case of sterilization which could be due to the heat induced degradation/modification of isoflavones.

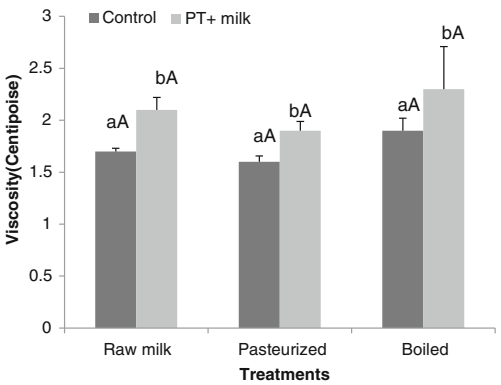

Fig. 2 Influences of addition *Pueraria tuberosa* on viscosity of milk. Data are presented as means±SEM (n=3). Similar superscripts in each row (**a**) and in each column (*A*) means not significant difference between groups (p>0.05). Statistical comparisons were made between samples using ANOVA single factor, PT - *Pueraria tuberosa*

Ethanol stability

Ethanol stability was measured at different concentrations of ethanol and it was observed that milk added with herb showed slightly less ethanol stability than control milk at pH 6.71 (Table 4). Analysis of variance revealed that there was a significant difference ($p<0.05$) in ethanol stability of control as compared to milk added with *Pueraria tuberosa* extract. In contrast to our observation, O'Connell and Fox (1998) noted that the freeze dried tea extract (green and black), cocoa powder and coffee caused a slight increase in the ethanol stability of milk throughout the pH range of 6.1 to 7.1. In another study by O'Connell and Fox (1999), there was no effect of addition of aloe vera extract, non-dialyzable red wine residue or aqueous methanol extracts of oak bark, oak leaves or coconut shell on ethanol stability of milk throughout the pH range 6.4 to 7.2. The possible mechanism of lowering of ethanol stability of milk added with *Pueraria tuberosa* could be related to destabilization of casein as a result of decrease in pH upon addition of herb extract.

Viscosity

Data with regard to the viscosity of control as well as milk added with *Pueraria tuberosa* extract are summarized in Fig 2. There were significant difference ($p<0.05$) observed between control and *Pueraria tuberosa* added milk subjected to different processing treatments. Viscosity of experimental samples increased significantly in raw milk, pasteurized milk and boiled milk as compared to control. No significant difference was however observed among treatments.

O'Connell and Fox, (1999) reported that addition of caffeic acid at 5.5 mM/lit had little effect on the relative viscosity of milk. The relative viscosity of milk, with or without caffeic acid at 5.5 mM/lit, was 1.69 Cp and 1.60 Cp, respectively. The possible increase in viscosity of *Pueraria tuberosa* added milk may be related to interaction of herb components with milk constituents and its destabilizing effect.

Conclusion

The additions of *Pueraria tuberosa* to milk at 0.4 % concentration altered the physicochemical properties of cow milk. Addition of herbs or its extracts to milk and subsequent processing treatments however poses a definite challenge as possibilities exist for varying degree of interactions among the major and minor biomolecules of milk and bioactive compounds in herbs. Such interactions could have beneficial effect but at times it may also lead to certain practical difficulties as they modify properties of the milk. Therefore, there is need to look into the possibilities of exploring the technological possesses which could minimise this interactions and still permit incorporation at higher concentrations.

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