Effect of *Asparagus racemosus* (shatavari) extract on physicochemical and functional properties of milk and its interaction with milk proteins

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Abstract The effects of interaction of *Asparagus racemosus* (shatavari) with milk constituents and physico-chemical and functional characteristics of milk was studied. Addition of freeze dried aqueous shatavari extract at a concentration of 1 g /100 ml of milk showed a decrease in pH, rennet coagulation time and an increase in acidity, viscosity and heat stability at maximum. The extract also imparted brown colour to milk and showed an increase in a^* (redness) and b^* (yellowness) values but a decrease in L^* (lightness) value. Proteins in milk were modified by reaction with shatavari extract. The derivatives formed were characterized in terms of SDS-PAGE. Electrophoretic pattern of sodium caseinate and whey containing 1% shatavari herb extract did not show any difference in band pattern i.e. there was no difference in mobility based on size of the proteins, but the intensity (width) of bands differed.

Keywords *Asparagus racemosus* · Shatavari · Cow milk · Physico-chemical properties · Heat stability · Rennet coagulation · Electrophoresis · Milk proteins

Introduction

Recently the universal trend has been a shift from synthetic to herbal medicine, which we can say ‘return to nature’. Herbs have been used as food and medicine for centuries. Medicinal plants serve as therapeutic alternative, safer choices, or in some cases, for effective treatment. *Asparagus racemosus* Willd. is one such important medicinal plant, which is regarded as a ‘*rasayana*’ (plant drugs promoting general wellbeing by increasing cellular vitality and resistance) in the Ayurvedic system of medicine (Goyal et al. 2003). It is an important medicinal plant of tropical and subtropical part of India up to an altitude of 1,500 m. The plant commonly known as Shatavari, Asparagus, Satavari or Satmul, belongs to the family Liliaceae. Shatavari is rich in active constituents such as steroidal glycosides, saponins, polyphenols, flavonoids, alkaloids (racemosol) and vitamins. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine. Traditionally, it is used as health tonic and common Indian home remedy used as a rejuvenator, promoter of strength, breast milk and semen. The medicinal/pharmacological value of shatavari root is attributed to the presence of steroidal saponins and sapogenins (Kapoor 2001). Fructo-oligosaccharides and other polysaccharides present in *A. racemosus* have been reported to be responsible for the immunomodulatory activity exhibited by it (Thakur et al. 2012). The root of shatavari is also used in the treatment of nervous disorders, dyspepsia, diarrhoea, dysentery, tumors, hyperdipsia, neuropathy and hepatopathy.

Food industries have rather high demand for the products that meet the consumer’s demand for a healthy life style. In this context, functional foods fortified with the plant ingredients play an important role. Herbal extracts in all their

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forms represent arguably the greatest potential for food formulator's quest for innovative functional food products. There are many companies already capitalizing on growing consumer acceptance of food and beverages containing herbal extracts (Rowan 2000), although the use of these extracts in milk and milk products is quite a recent development. Milk is also one of the most widely consumed foods in the world and is an ideal vehicle for fortification with these nutraceuticals. However, it is imperative to investigate the physicochemical and functional properties of herb extract-milk system and interaction of herbal extract with milk proteins to establish efficacy of nutraceuticals in dairy foods. The purpose of this study was to determine the effect of shatavari extract on physicochemical and functional properties of milk as well as to determine the interaction between milk proteins and herbal extract by SDS-PAGE.

Materials and methods

Milk supply Raw cow milk obtained from the cattle yard of National Dairy Research Institute, Karnal, India, was defatted by cream separator (Kamdhenu, KD-60E, Benny, India). Both whole milk and skim milk were pasteurized at 72 °C/15 s and boiled for 2 min.

Plant material and preparation of extract The roots of *A. racemosus* were bought from local market and authenticated. They were deposited in the departmental herbal drug museum of the Pharmacognosy Division, National Botanical Research Institute, Lucknow, India for future reference. Air-dried (40 °C–50 °C) and powdered roots (50 g) were extracted with hot water (5×400 mL) by continuous heating on a water bath at 100 °C for 5 h each time. The extracts were pooled together, filtered and concentrated under reduced pressure at 60 °C–65 °C by rotary evaporation (Büchi, USA), and lyophilized (Freezone 4.5; Labconco, USA) under high vacuum (133×104 mbar) at −40 °C±2 °C to yield the freeze dried aqueous extract of shatavari (17 g).

Influence of shatavari extract on some physicochemical and functional properties of milk Preliminary trials were conducted to optimise the levels of aqueous extract of shatavari in milk by adding it at three different levels (1, 1.5 and 2 %). One per cent level in milk was observed to be an optimum level on preliminary sensory evaluation for colour, flavour and texture by 9-point hedonic scale (Piggott 1984). The contents of total solids, fat, lactose as well as ash in control and experimental milk samples were estimated following the methods as described in ISI (1981). Samples were also analysed for protein contents by micro-Kjeldahl method (AOAC 2000).

Titrateable acidity and pH Titrateable acidity and pH of control and experimental milk samples were determined by the method described in ISI (1981).

Colour measurement Colour of the control and experimental cow milk, pasteurized milk and boiled milk samples was measured using a Colourflex (Hunterlab, Reston, Virginia, USA) along with the universal software. The light source was dual beam xenon flash lamp. Data was received through the software in terms of L* (Lightness) ranging Zero (black) to 100 (white), a* (Redness) ranging from +60 (red) to −60 (green) and b* (Yellowness) +60 (yellow) to −60 (blue) of the International colour system.

Viscosity Viscosity of control and experimental milk samples (20 °C) was determined using Fungi lab rotational viscometer (Visco star plus, Spain) which is an open, concentric measurement system and allows measurement by immersion. The measuring head and measuring tube are rigidly coupled; the measuring unit is driven by a DC motor. Viscometer fitted with spindle TL-5 and adjusted the rpm to 50. A built-in microprocessor calculates the value for the viscosity (in terms of centipoise).

Heat stability Samples of control and experimental skimmed milk were adjusted to pH values in the range 6.3–7.1 with 0.1 N NaOH or HCl and the heat stability determined by the subjective method of Davies and White (1966).

Rennet coagulation Samples of control and experimental cow milk, skimmed milk, pasteurized milk samples (5 ml) placed in screw capped test tubes, were tempered for 10 min at 30 °C. Commercial calf rennet was added to each milk samples. The tubes were placed in thermostatically controlled water bath at 30 °C and rennet coagulation time was determined according to the method of Berridge (1952).

Interaction studies by Electrophoresis Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE)

Table 1 Proximate composition of control and shatavrai supplemented milk

Constituent, %	Control	SSM
Fat	4.46±0.03 ^a	4.40±0.03 ^a
Total solids	11.6±0.03 ^a	12.48±0.02 ^b
Protein	3.32±0.04 ^a	3.38±0.02 ^a
Lactose	4.88±0.06 ^a	4.98±0.03 ^a
Ash	0.699±0.01 ^a	0.710±0.03 ^a

Data expressed as mean ± SE, n=3. Values with different superscripts (a, b) in a row are statistically significant at $p<0.05$

SSM shatavari supplemented milk

Table 2 Effect of addition of shatavari extract on pH, acidity and viscosity of milk

System	pH	Acidity (% Lactic acid)	Viscosity (centipoise)
Control	6.59±0.003 ^a	0.152±0.003 ^a	2.5±0.03 ^a
SSM	6.51±0.003 ^a	0.169±0.008 ^a	3.0±0.03 ^b

Data are presented as mean ± SE ($n=3$). Mean in a column with different superscripts (a, b) are significantly different ($P<0.05$) from each other

SSM shatavari supplemented milk

(Laemmli 1970) was performed in order to know the interaction between the milk protein and shatavari herb extract. SDS-PAGE was run for both control and shatavari added milk samples, but the visibility of bands was poor and overlapping each other because of the interfering substances in the milk. Hence, sodium caseinate and whey were prepared from the milk (Mulvihill 1989) to study the interactive effect. The gels were analysed by using ImageAide gel analysis software (Spectronics Corporation ImageAide for Windows) and the densitograms were then drawn. The results were expressed in terms of height and raw volume (Area under the curve) of each band in the lane. Molecular weight standards (205 KDa to 3.5 KDa) were obtained from Genei, Bangalore.

Statistical analysis The studies were replicated three times. 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 $p<0.05$. *t*-Test was used to determine the main effects of treatments.

Table 3 Instrumental colour parameters of control and shatavari supplemented milk

	Raw milk	Pasteurized milk	Boiled milk
L* (Lightness)			
Control	83.5±0.10 ^a	83.6±0.17 ^a	84.5±0.33 ^a
SSM	77.1±0.03 ^b	77.4±0.26 ^b	78.5±0.15 ^b
a* (Redness)			
Control	-2.3±0.28 ^a	-2.2±0.35 ^a	-2.5±0.83 ^a
SSM	1.0±0.08 ^b	0.83±0.07 ^b	0.71±0.11 ^b
b* (yellowness)			
Control	11.3±0.11 ^a	11.0±0.32 ^a	11.3±0.56 ^a
SSM	14.4±0.16 ^b	14.2±0.56 ^b	14.2±0.27 ^b

Data are presented as mean ± SE ($n=3$). Mean in a column with different superscripts (a, b) are significantly different ($P<0.05$) from each other

SSM shatavari supplemented milk

Fig. 1 Effect of addition of shatavari extract at 1 %, w/v, on the HCT-pH profile of skim milk. Data presented as mean±SE ($n=3$)

Results and discussion

Physicochemical analysis Fat, protein, lactose and ash levels of shatavari supplemented milk did not differ significantly ($P>0.05$) compared to control (Table 1). Significant difference was observed between the total solids content of shatavari supplemented milk and control.

There was an apparent difference in pH and acidity of control whole milk and milk added with shatavari extract (Table 2). However, this difference was non-significant ($P>0.05$) for both pH and acidity. The decrease in pH of shatavari added milk might be due to presence of acidic components (ascorbic acid) in the herbal extract. There was a significant increase in viscosity of shatavari supplemented milk as compared to control (Table 2). Increase in viscosity of shatavari supplemented milk might be due interaction of milk constituents with phytochemicals of the extract namely fructo-oligosaccharides and other polysaccharides present in it. The changes in lightness L^* (black/white), a^* (redness/greenness) and b^* (yellowness/blueness) values were significant for both control and shatavari supplemented milk (Table 3). Addition of shatavari extract imparted brown colour to milk and it mainly affects the lightness (L^*) and redness (a^*) value. Shatavari supplemented milk showed an increased a^*

Table 4 Effect of addition of shatavari extract on rennet coagulation time (RCT) of milk at 30 °C

Sample	RCT, min	
	Control	SSM
Whole milk	12.3±0.07 ^a	4.3±0.02 ^b
Skim milk	32.5±0.03 ^a	8.3±0.03 ^b
Pasteurized whole milk	25.3±0.05 ^a	6.3±0.05 ^b
Pasteurized skim milk	63.4±0.06 ^a	9.5±0.08 ^b

Data presented as mean ± SE ($n=3$). Mean in each row with different superscripts (a, b) are significantly different ($p<0.05$) from each other

SSM shatavari supplemented milk

Fig. 2 SDS-PAGE pattern of sodium caseinate and whey added with shatavari separated on 15 % gel. Lane 1 Sodium caseinate, Lane 2 Sodium caseinate + shatavari extract (1 %), Lane 3 Molecular weight standards ranging from 205 KDa to 3.5 KDa, Lane 4 Whey, Lane 5 Whey + shatavari extract (1 %)

(redness) and b^* (yellowness) values with a decrease in L^* (lightness) value as compared to control. Maillard products during heat treatment of milk may also be responsible for the colour changes. The intensity of heating resulted in an increase of L^* value in both the control and shatavari supplemented milk. The L^* value decreased in the order of boiled milk > pasteurized milk > raw milk in both control and shatavari supplemented milk, but had no significant effect on b^* and a^* values.

Heat stability The effect of addition of aqueous shatavari extract (1 %) on heat stability of raw skimmed milk is shown in Fig. 1. In shatavari supplemented milk, there was no improvement in heat stability up to a pH of 6.70 and the maximum heat stability was observed at pH 6.8. The shatavari extracts markedly increased heat stability of skimmed milk at pH 6.8, mainly by slight shift in the HCT-pH profile and enhanced stability at the maximum as compared to control milk. O'Connell and Fox (1999a,b) reported that phenolic compound-rich extracts from a variety of plant sources, e.g., tea, coffee, cocoa, wine, oak and pine bark and aloe vera, and purified phenolic compounds, e.g., caffeic acid, 1,2-dihydroxynaphthalene, epigallocatechingallate and 3,4-dihydroxybenzaldehyde markedly increased heat stability of milk and

concentrated milk. Stabilising effect of phenolic compounds is related to their ability to thermally oxidise to quinones, which are extremely electrophilic and interact with nucleophilic amino acid residues, e.g., lysine and cysteine, to maintain micellar integrity, which is compromised on heating. The increase in heat stability of shatavari supplemented milk might be due to the interaction of milk proteins with phytochemicals such as phytosterols, saponins (shatavarins I–IV), polyphenols, ascorbic acid, alkaloids and flavonoids which are present in the shatavari herb extract.

Rennet coagulation time The data in Table 4 represents the rennet coagulation time (RCT) of control and experimental milk. Addition of shatavari extract decreased the RCT of milk as compared to control in both whole milk and skim milk. The reaction proceeds faster with the formation of firm clots as the pH was lowered below that of milk. Decrease in pH probably also affected the stability of caseinate particles directly as well as indirectly by release of calcium ions from dissolved and colloidal complexes as the pH is lowered. Grandison et al. (1984) and Ostensen et al. (1997) found that milk pH affects predominantly the rennet coagulation time. Shatavari supplemented milk showed a decrease in pH compared to control milk and hence, decreased the rennet coagulation time.

Shatavari supplemented skim milk and control skim milk showed a significantly higher rennet coagulation time than the corresponding whole milk samples. The results indicated that fat influences the first steps of union of the destabilised micelles. The influence of heat treatments on the changes in the fat globule membrane has been reported. Some authors (Sharma and Dalgleish 1993; Corredig and Dalgleish 1996) have described that whey proteins are adsorbed to fat globule membrane after milk was heated. The possible changes in the fat globule membrane during heat treatment could improve the destabilised micelles aggregation in whole milk. On the other hand, the aggregation of denatured whey proteins to fat globule membrane could reduce the aggregation of these proteins with the κ -casein, influencing directly the enzymatic rennet action and, perhaps, indirectly the first steps of micelles aggregation. Since skim milk used in this study was mostly free from the fat globules. Hence, the whey proteins complexed with κ -casein and thus delayed the rennet coagulation time.

Table 5 ImageAide readings of height and raw volume (area under the curve) of different bands of each lanes of SDS-PAGE Gel

Track No	Lane 1		Lane 2		Lane 4		Lane 5	
	(sodium caseinate)		(sodium caseinate + Shatavari)		(Whey)		(Whey + Shatavari)	
	Height	Raw Volume	Height	Raw volume	Height	Raw Volume	Height	Raw Volume
1	38.73	324756	53.34	1058822.3	59.94	2036060	76.48	3164788
2					48.55	833479	66.62	1703345

Pasteurized whole milk and skim milk (shatavari supplemented milk as well as control) samples showed a higher rennet coagulation time than the corresponding unpasteurized samples. Milk coagulation is strongly dependent on the temperature. The velocity of coagulum formation increases progressively from 20 to 40–42 °C, but at higher temperatures, the coagulation process slows down. It has been observed that the temperature of the milk affects protein aggregation rate to a large extent and that increased temperature increases the rate of gel firming. Sharma and Dalgleish (1993) have described that formation of a complex between proteins from fat globule membranes and whey proteins at a high temperature could influence aggregation of whey proteins with κ -casein and consequently the enzymatic effect of rennet on κ -casein.

Electrophoresis: SDS-PAGE To investigate the interactive effect of milk proteins with shatavari extract, sodium caseinate and whey were used for SDS-PAGE electrophoresis. SDS-PAGE was applied for the molecular weight determination and change in band intensity was measured using ImageAide gel analysis software. Sodium caseinate and whey proteins were resolved on 15 % SDS gel. Figure 2 shows the electrophoretic patterns of sodium caseinate and whey containing 1 % shatavari herb extract. It did not show any difference in band pattern i.e. no difference in mobility based on size of the proteins, but the intensity (width) of band differs. Gel analysis software ImageAide, measured the height and area under curve (raw volume) of each lane of SDS-PAGE gel represented in Table 5. There was an increase in the raw volume and height of shatavari added sample as compared to the control. The increase in bandwidth might be due interaction between the phytochemicals (polyphenol, flavonoids, saponins, alkaloids) and milk proteins (casein and whey proteins).

These results are in line with those obtained by Rawel et al. (2001) who studied the interaction of plant phenols with whey proteins. The formation of high molecular weight fractions was documented with SDS-PAGE. Especially the derivatives of chlorogenic, caffeic, gallic acid and p-quinone showed an increase in molecular weight of β -lactoglobulin fraction from 18,300 to 20,000 Da. Spencer et al. (1988) reported the ability of phenolic compounds to interact with (and even precipitate) proteins, particularly proline rich proteins such as salivary proteins and caseins. The principal cohesive forces under mild conditions appear to be hydrophobic and hydrogen bonding (Haslam and Lilley 1988).

Conclusion

The present studies revealed that shatavari extract plays a certain role in altering the functional properties and stability of milk fortified with it. Shatavari added milk showed

decrease in pH, rennet coagulation time, L* value and increase in acidity, viscosity, heat stability, a* and b* value. Electrophoretic patterns of sodium caseinate and whey containing shatavari extract showed an increase in band intensity due to interaction between the phytochemicals and milk proteins. These results will, therefore, be useful in acquiring basic knowledge for the development of herb fortified functional dairy foods.

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