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CONTENTS

Incorporation of Extracts of Pulse Sprouts in Market Milk

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ABSTRACT

Cow milk is a good source of proteins, fat, ash and lactose present in balanced proportion. It is also a source of calcium and vitamin A. However, its value can further be buttressed by fortification with other nutritionally rich components such as sprout extracts. Freshly sprouted pulses are rich in nutrients; especially they are rich in enzymes, digestible energy, bioavailable vitamins, minerals, amino acids, proteins and phytochemicals. Direct consumption of sprouted beans is limited by beany flavour. Many consumers, especially children, may not relish the typical beany flavor of the sprouts. In this regard, fortification of milk with the sprout extracts will be helpful for consumers nutritionally. In this project, sprout extracts of green gram, Bengal gram and horse gram were added directly into milk. The various levels of sprout extracts' incorporation not resulting in detectable beany flavours were determined by sensory evaluation. The extract levels in milk that can be used without perceivable beany flavour were: 0.5% Bengal gram sprout extract; 1% horse gram extract in plain milk; these levels in flavoured milk were 1.5% and 2%, respectively. The acceptable levels of autoclaved – sprout extracts were 5% and 5% in plain milk, and 15% and 15% extracts of Bengal gram and horse gram sprouts in flavoured milk, respectively. Raw-green gram sprout extract was not recommended in plain milk, but up to 25% of autoclaved-sprout extract could be incorporated in plain milk and 30% in flavoured milk without detection of beany flavour. These results may help in incorporation of various bean extracts in market milk.

Key words: Milk, sprout extracts, sensory evaluation, Bengal gram, green gram, horse gram

INTRODUCTION

Market milk is defined as milk that is produced in conformity with regulations of the public regulatory or health authority for disposition by sale. It is consumed by most of the population in India. Bovine milk is consumed by humans since time immemorial for its nutrients and health beneficial properties. Of late, efforts are being made to replenish milk with certain factors like vitamins, minerals etc. which it is supposedly is deficient in (Miller et al., 2007; Preedy et al., 2013). There is a scope to fortify milk with even vegetable sources like bean sprouts. The sprouting process helps in the synthesis of A, B and C vitamins. Among the sprouts Bengal gram, green gram and horse gram are commonly used. Sprouting is the practice of germinating seeds to be eaten either raw or cooked. They are a convenient way to have fresh vegetables, in any season and can be germinated at home or produced industrially. Sprouts are believed to be highly nutritious and rich in enzymes which promote good health. They are a prominent

ingredients of the raw food diet and common in Eastern Asian cuisine.

Pulse sprouts are having good quality proteins with low fat and high fibre. By a process of natural transmutation, sprouted food acquires vastly improved digestibility and nutritional qualities when compared to non-sprouted ones. Sprouts have several other benefits. They supply food in pre-digested form, that is, the food which has already been acted upon by the enzymes and made to digest easily. During sprouting, much of the starch is broken down into simple sugars such as glucose and sucrose by the action of the enzyme 'amylase'. Proteins are converted into amino acids and amides. Fats and oils are converted into more simple fatty acids by the action of the enzyme lipase. Thus, sprouts are an extremely inexpensive method of obtaining a concentration of vitamins and enzymes. Germination of mung beans increases vitamin C content in mung bean sprouts. On fresh weight basis, one serving of mung bean sprouts (about 104 g) provides 21.6 mg of vitamin C, which could meet 36% of daily value. Germination also increases total phenolic compounds and total flavonoids in mung bean sprouts in a time-dependent manner, up to 4.5 and 6.8 times higher than the original concentration of mung bean seeds, respectively. According to Guo et al. (2012), the total antioxidant activity of mung bean sprouts was increased by 6 times higher than that of mung bean seeds. Khattak et al. (2007) reported that vitamin C content increased during germination of chickpeas.

About 45% of bovine milk produced in India is consumed as liquid milk (Dairy India, 2019). Fortification of milk with nutrients will have health benefits to consumers. Therefore, fortification of milk with minerals and vitamins has been recommended (Sumit Arora, 2011; Khadgawat et al., 2013) and being followed by the industry. Similarly, it is presumed that fortification of milk with sprout extracts will also accrue benefits to consumers.

MATERIALS AND METHODS

Ingredients and equipment

The fresh raw milk was obtained from Experimental Dairy, NDRI, Bangalore and it was standardized to required level of fat using skim milk. Bengal gram, horse gram and green gram were purchased from local market to prepare sprouts. All glassware used in the experiments were washed with detergents and dried before use. The chemicals of Ranchem brand, AR grade were used in the analysis of the products. Sugar and cocoa powder used in the study were procured from local market.

Methods

Preparation of sprouts

Good quality seeds were selected, washed thoroughly in potable water and then soaked overnight in a vessel in potable water (seeds to water ratio 1:4). The vessel was kept covered with cheesecloth or wire screening and soaked for 10 to 12 hours. On the following morning, the seeds were rinsed and the water drained off. The swollen seeds were kept in the vessel and covered with lid allowing some air circulation. The vessel was left in a cool dry place for 2-3 days. The seeds germinated and became sprouts in two or three days from commencement of soaking.

Preparation of sprouts extract

Raw-sprout extract

Sprouts were washed in potable water and the water drained. Hundred gm of sprouts were taken in a mixer jar vessel and added with 100 ml potable water. The sprouts were ground along with the water to form a mixture. The mixture was filtered through muslin cloth to obtain the sprout extract.

Autoclaved-sprout extract

Sprouts of 125gm were taken in a container, added with little quantity of water and placed in an autoclave. It was autoclaved till pressure reached 10psi. The autoclaved-sprouts were washed in potable water two times. During washing the outer coat was removed automatically and discarded along with wash water. Sprouts were taken in a mixer jar, added with equal quantity of water and then ground to form a uniform mixture. The mixture was filtered through muslin cloth to obtain autoclaved-sprout extract.

Incorporation of extract into milk

Raw-sprout extract

Raw-sprout extracts were incorporated into milk @ 0.5, 1.0 and 1.5 ml green gram extract per 100 ml toned milk; 0.5, 1.0, 1.5 and 2.0 ml Bengal gram extract per 100 ml of toned milk, and 0.5, 1.0, 1.5, 2.5 ml horse gram extract per 100 ml of toned milk. All the milk samples taken in glass beakers were pasteurised by heating to 63^{o}C and holding at that temperature for 30 min in a water bath. In other batches, all the milk samples were added with 2% cocoa powder, sugar @ 8% and mixed thoroughly. The samples were heated to about 80^{o}C. All the milk samples were cooled to ambient temperature (28^{o} – 30^{o}C) and sensorily evaluated for presence of any beany flavour, and overall acceptance on nine – point Hedonic scale.

Autoclaved-sprout extract

Autoclaved-sprout extracts were incorporated into milk @ 20, 25, 30 and 35 ml green gram extract per 100 ml toned milk; 5, 10, 15 and 20 ml Bengal gram extract per 100 ml of toned milk, and 5, 10, 15 and 20 ml horse gram extract per 100 ml of toned milk. All the milk samples taken in glass beakers were pasteurised by heating to 63^{o}C and holding at that temperature for 30 min in a water bath. In other batches, all the milk samples were added with 2% cocoa powder, sugar @ 8% and mixed thoroughly. The samples were heated to about 80^{o}C. All the milk samples were cooled to ambient temperatures and sensorily evaluated for presence of any beany flavour, and overall acceptance on nine – point Hedonic scale.

Evaluation of sprout extract incorporated milk

The prepared samples were evaluated for the following parameters:

Sensory acceptance

Taste panel consisting of scientists and PG students and faculty of dairy technology performed the sensory evaluation of the milk samples. Taste panels were organized around 12 noon or between 3 -5 PM every time. The milk samples added with sprout extracts were taken in glass containers and served to 6-8 judges who were explained the nature of the experiment. The samples were evaluated for presence of any beany flavour and overall acceptance. The judges were asked to score the acceptance level on a 9-point Hedonic scale wherein a score of 1 indicated dislike extremely and a score of 9 indicated like extremely (Amerine et al., 1965). The sensory evaluation was

conducted in the laboratory under fluorescent lights. Saline water was provided to rinse the mouth in between the samples. A score of 7.0and above was considered acceptable.

Physico-chemical analysis

Specific gravity

Specific gravity bottle (25 ml) which was thoroughly washed and dried was filled with distilled water and stopper placed allowing overflow of the water and care was taken not to leave any air bubbles in the water sample. The bottle was then wiped with tissue paper from outside and the bottle with the water was weighed accurately. After weighing, the bottle was emptied and rinsed with milk sample. Now, the bottle was filled with milk sample as above and the weight recorded. Temperature of the sample was about 30°C. Specific gravity was determined as follows:

Specific gravity = weight of sp gr bottle with milk sample / weight of sp gr bottle with

distilled water

Viscosity

The procedure described by Sudha et al. (2019) was followed. Ostwald viscometer (Vensil Make) was thoroughly cleaned in detergent solution and rinsed with distilled water. With the help of a pipette, 13 ml of distilled water was carefully transferred to wider stem of the viscometer. From the other end, suction was applied so that the water rose through the capillary and into the bulb chiseled with lower and upper marks. Suction was stopped after the liquid level was above the upper mark demarcated on the bulb. The suction was removed and liquid allowed to flow downwards through the bulb till it passed through the upper mark and then lower mark. The time elapsed between the upper and lower marks was recorded. These steps were repeated thrice with distilled water and average time elapsed was recorded in seconds (tw). The viscometer was then emptied and rinsed with milk sample. Then same procedure was followed as above replacing the water with milk samples. The average time elapsed (tm) was recorded in seconds. All the recordings were done at about 30°C. Instrument constant (K) was calculated by: 1/tw. Then kinematic viscosity of milk (V) was calculated by: V = K.tm expressed in centi Stokes (cS).

Protein

The protein content of milk was estimated by Pyne's titration method (Prasadarao, 1991) with a Pyne's constant of 1.7. Ten ml of milk was taken in a 100 ml conical flask, added with 0.2 – 0.5 ml pottasium oxalate solution and kept for 2 – 3 min undisturbed. Then 4-5 drops of phenolphthalein were added and titrated against 0.1N NaOH to light pink end point. After that, 2ml formaldehyde was added resulting in discoloration of the flask contents which was again titrated against 0.1N NaOH to light pink colour end point. Protein content of milk was calculated by:

Protein % = V x 1.7

where 1.7 – Pyne's constant; V – second titre value, ml

RESULTS AND DISCUSSION

Sensory analysis of sprout extract added milk samples

Based on their ranking, sample having highest score was considered acceptable. Sample having detectable beany flavor was rejected. In green gram sprout extract added milk, a comparison was made between raw and

flavored milks with raw and autoclaved extracts. The green gram raw extract milk sample was rejected due to its pronounced beany flavour. Beany flavor is attributed to several aldehyde and ketone compounds formed as a result of action of lipoxygenase on unsaturated fatty acids in pulses (Roland et al., 2017). The acceptance scores of green gram sprout extract added milk samples are given in Table-1. It may be seen that raw extract addition resulted in more beany flavor than autoclaved extract. Control sample in which no sprout extract was added scored an overall acceptance score of 8.5 indicating a very good acceptance. However, addition of raw-green gram sprout extract drastically reduced the acceptance score because of beany flavour. Raw extract added at a low level of 0.5% itself produced perceivable beany flavor in milk, hence the milk was awarded lower scores of acceptance. Even addition of cocoa powder could not mask the beany flavor, hence addition of raw green gram extract was not recommended. However, on addition of autoclaved-sprout extract did not result in beany flavor up to 25% of addition (acceptance score 8.0), beyond which beany flavor could be detected. However, addition of cocoa powder masked the benay flavor and the extract could be added up to 30% of the milk (acceptenac score 8.0).

In case of Bengal gram raw-sprout extract, the beany flavour was detected beyond 0.5% level of incorporation, whereas on addition of cocoa powder, the beany flavor could be masked up to 1.5% addition (score 7.0) (Table-2). In case of autoclaved-sprout extract, the acceptable limit of incorporation was 5% and in case of cocoa flavoured milk it was only 15% beyond which beany flavor was perceivable.

In case of horse gram raw-sprout extract, 1% addition was found to be acceptable in milk without perceivable beany flavor (score 7.0) (Table-3). In presence of cocoa powder, the addition of the Bengal gram sprout extract could be enhanced to 2%, the acceptance score being 7.5. Using autoclaved-sprout horse gram extract, higher levels could be added. While autoclaved extract addition was acceptable up to 5%, the same in presence of cocoa powder was limited up to 15%.

Thus, it may be found that the acceptable raw-sprout extract levels that could be used in plain pasteurised milk were 0.5 – 1.0% and the same in presence of cocoa powder was 1.5 – 2%. Whereas the autoclaved-sprout extracts could be added @ 5 – 25% in plain milk and 15 – 30% in case of cocoa flavoured milk. The results indicated that raw-sprout extracts caused intense beany flavor in plain milk limiting the addition level to 1% which slightly increased to 2% in case of cocoa flavoured milk. This shows that cocoa powder addition had little influence in masking the beany flavor. From the observations, it can also be observed that on autoclaving the beany flavor of the extracts could be subsided and more amount of the extract could be incorporated into the milk. In this case, cocoa powder could also significantly mask the beany flavor as indicated by the higher acceptable levels of extract addition in cocoa flavoured milk. The beany flavor of the sprouts can be attributed to several volatile compounds and on autoclaving the beany flavor evaporates. In general, germination and cooking processes are known to decrease the beany flavor by inactivation of enzymes (Roland et al., 2017).

Physico-chemical properties of sprout extract incorporated milk

The extracts prepared from three pulse sprouts namely green gram, Bengal gram and horse gram were

incorporated into toned milk at the acceptable levels and the milk samples were pasteurized. The cooled, pasteurized milk samples were evaluated for protein content, viscosity, specific gravity and sensory acceptance. The results obtained are as follows.

Protein

Protein content of sprout extract added milk remained same or slightly decreased when compared to control sample of milk. The milk sample in which raw-sprout was added had almost same protein content (3.18 - 3.2%) as that of control (3.2%), but samples with autoclaved sprout extract possessed slightly lower protein contens (2.82 – 3.13%%) (Table-4). Among the three pulse sprouts, green gram sprout added milk had least protein content (2.82%). It was further observed that the protein content of sprout extract added milk samples were not higher than that of control sample, because the protein content of the extract itself was lower than that of milk causing a dilution effect, especially in case of autoclaved-sprout extract samples. This may be attributed to slightly higher moisture content of autoclaved-sprouts. Singhal et al. (2011) reported protein content of milk as 3.28% which is comparable with the values obtained for control sample in the present study.

Viscosity

Viscosity of raw extract added milk samples was slightly higher than autoclaved-sprout extract added milk samples. This is because of higher moisture absorbed by sprouts during autoclaving. In general, viscosity varied from 1.89 – 2.01 cS in case of sprout extract added samples whereas control milk had a viscosity of 1.99cS (Table-4). In general, it may be said that there was no much variation in viscosity of milk by addition of sprout extracts. Deeth and Lewis (2017) reported viscosity of milk as 2.0 cP which is equal to 1.95 cS (taking specific gravity of milk as 1.028) and comparable with the value obtained in the present study. Proteins are known to significantly contribute to the viscosity of milk. Fat is the influential constituent followed by protein on viscosity of milk (Webb and Johnson, 1965; Sudha et al., 2019). They bind water (Kneifel et al., 1991) and thus enhance viscosity. Sudha et al. (2019) reported the viscosity of the flavoured milk as 1.95, 1.98 and 2.01 cS respectively when 0.5, 1.0 and 1.5 gm lemongrass leaves were used for extraction.

Specific gravity

The specific gravity of normal milk and sprout extract added milk was nearly equal ranging from 1.026 – 1.031 (Table-4). Yoganandi et al. (2014) reported specific gravity of pooled cow milk as 1.029 which is closer to the values reported in the present study. Sudha et al. (2019) reported the specific gravity values of the flavoured milk as 1.075, 1.076 and 1.080, respectively for lemongrass leaves of 0.5, 1.0 and 1.5 gm.

CONCLUSION

In this study, Bengal gram, green gram and horse gram sprout extracts in both raw and autoclaved forms were incorporated into milk. Raw green gram extract resulted in pronounced beany smell and it was rejected. Bengal gram and horse gram extracts could be added up to 0.5% -1.0% level in pasteurized milk and 1.5% -2.0% in cocoa flavoured milk. However, autoclaved extract could be used 5-15% in pasteurised milk and cocoa flavoured milk. Use of cocoa suppressed beany flavour. So, autoclaved-sprout extracts could be added to flavoured milks than in

pasteurized milk. These results are helpful to dairy industry to fortify market milk with sprout extracts.

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Table-1:Sensory acceptance of market milk incorporated with green gram sprout extract

D – Detectable, ND – Not detectable

Percentage	Plain milk		Flavoured milk	
	Score*	Beany Flavor	Score*	Beany flavor
Raw-sprout extract				
0	8.5	-	8.5	-
0.5	5.0	D	6.0	D
1	3.0	D	3.0	D
Autoclaved-sprout extract				
20	8.0	ND	8.4	ND
25	8.0	ND	8.3	ND
30	4.0	D	8.0	ND
35	4.0	D	6.0	D

Table-2:Sensory acceptance of Bengal gram sprout extract incorporated milk

Percentage	Plain milk		Flavored milk	
	Score	Beany Flavor	Score	Beany flavor
Raw-sprout extract				
0	-	-	-	-
0.5	7.5	ND	7.9	ND
1	6	D	7.5	ND
1.5	4	D	7	ND
2	4	D	5	D
Autoclaved-sprout extract				
5	8	ND	8	ND
10	5	D	7.6	ND
15	5	D	7	ND
20	3	D	4	D

D – Detectable, ND – Not detectable

Table-3:Sensory acceptance of horse gram sprout extract incorporated milk

Percentage	Plain milk		Flavoured milk	
	Score	Beany flavor	Score	Beany flavor
Raw-sprout extract				
0				
0.5	7.5	ND	7.8	ND
1	7	ND	7.5	ND
1.5	5	D	7.5	ND
2	4	D	7.5	ND
2.5	4	D	5	D
Autoclaved-sprout Extract				
5	7.8	ND	8	ND
10	5	D	8	ND
15	4	D	7	ND
20	4	D	4	D

D – Detectable, ND – Not detectable

Table-4:Physico-chemical properties of market milk incorporated with sprouted gram extracts

Property	Normal milk	Plain milk incorporated with					
		GG raw-sprout extract@0.5%*	GG autoclaved- sprout extract @25%	BG raw-sprout extract@0.5 %	BG autoclaved- sprout extract @5%	HG raw-sprout extract@1 %	HG autoclaved- sprout extract @5%
Protein, %	3.2	3.18	2.82	3.20	3.13	3.2	3.1
Viscosity at 30°C, cS	1.99	2.0	1.90	2.01	1.89	2.0	1.90
Specific gravity	1.029	1.029	1.027	1.029	1.026	1.030	1.025

Note: GG – Green gram; BG – Bengal gram; HG – Horse gram
* Not acceptable because of beany flavour