I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS) Rating : 3. 03

To Study the Preparation of Ready to Serve Beverage from *Trifolium Alexandrinum*

Prity Pant[1], Shanky Bhat[1], Kritika Tripathi[1] and Sonali Nagar[1]

ABSTRACT: *Berseem is the most important fodder crop of India. It is very nutritive, easily digestible and highly palatable to all farm animals. Since it is a leguminous crop, also enriches soil fertility. Trifolium alexandrinum is a herb belonging to the family Fabacaceae. It is cultivated as a fodder crop for cattle. It was selected because it is fast-growing, resistant to pollution loads, produces high biomass and above all offers multiple harvests in a single growth period. Foods of animal origin especially dairy products are major sources of conjugated Linoleic acid (CLA) for humans and are reputed to have therapeutic health values including anti carcinogenic properties. The present study was undertaken with a view to optimize the percentage of berseem and spinach for RTS preparation keeping rest components constant, as well as to study storage analysis (at room temperature (25 ± 5°C) and refrigeration (3 ± 1°C) temperatures) of the beverage till it's safe to consume. On the basis of above findings, it may be concluded that 40: 40 concentration of berseem and spinach was most suitable for the preparation of RTS beverage and can be stored satisfactorily for a period of 8 weeks at refrigeration temperature (3 ± 1°C) with addition of sodium benzoate. Further RTS beverage which was stored at refrigeration temperature was more acceptable than beverage stored at room temperature (25 ± 5°C).*

Keywords: *Beverages, CLA, Berseem, Nutrition, Storage.*

INTRODUCTION

Beverages are very well-liked across the country and people from all age groups drink either hot or cold beverages regularly. There have been variety of soft drinks presently produced in the country, *e.g.* Still beverages containing fruit juice/pulp and soda water,sweetened carbonated (aerated) soft drinks. Among these, the share of vegetable juice based beverages is presently quite small as compared to synthetic carbonated drinks. Beverages based on fruits and vegetables products are currently receiving considerable attention as their market potential is growing. Besides being delicious, these beverages are highly nutritious. Gradually there is a distinct shift towards fruit/vegetable juice based beverages for obvious advantages of the higher nutritional value over the synthetic aerated waters.

Fruits and vegetables are a rich source of vitamins, sugars, and minerals. However, some vegetables and fruits have an off flavor and bitterness although they are an excellent source of enzymes, vitamins, and minerals. So, mixing of two or more fruit and vegetable juices with spices extract for the preparation of nutritive ready-to-serve (RTS), beverages is thought to be a economic and convenient alternative for utilization of these fruits and vegetables. Moreover, one could think of a new product development through mixing in the form of a natural health drink, which may also serve as an appetizer. The present review focuses on the blending of vegetable, fruit, fodder and spices in appropriate proportions for the preparation of natural vegetable and fodder based nutritive beverage.

Berseem clover (*Trifolium alexandrinum*) that's the origin is not very clear, since the original wild parent seems to have now become extinct. Some authorities believe that berseem clover probably originated in Syria and was first introduced into Egypt in about the 6th century. It was introduced into India in 1904. Berseem clover is also known as Egyptian and Alexandria clover. Berseem clover seeds have been

[1] School of Biotechnology, Gautam Buddha University, Greater Noida, U.P.

long been utilized in the South for forage. Its forage contains approximately from 18 to 28 percent crude protein, which is very comparable with alfalfa. It is also gaining huge popularity in the mid-west as a cover crop. It has incredible potential for providing high quality forage and can help in improving soil conditions as a green manure crop because of its fast growth characteristics and capacity to fix nitrogen[1]. Foods of animal origin especially dairy products are major sources of conjugated Linoleic acid (CLA^2) for humans and are reputed to have therapeutic health values including anti-carcinogenic properties.

It may be particularly useful in places where there is inadequate nutrition, which could lead to nutritional deficiency diseases.

Spinach (Spinacia oleracea) is a member of the Chenopodiaceae (Goosefoot family), which is an excellent source of Vitamin K, Vitamin A, Manganese, Folate, Magnesium, Iron, Vitamin C, Vitamin B2 (Riboflavin), Calcium, Potassium, and Vitamin B6. The strong natural mineral and vitamin content in spinach can be an effective source of healing for a multitude of health ailments.

Objectives-This study was conducted

- To study and prepare ready to serve beverage from berseem and spinach.
- To optimize the berseem and spinach ratio in RTS beverage keeping rest components constant.
- To perform the storage analysis at room temperature (25 ± 5°C) and refrigeration (3 ± 1°C) temperature.

MATERIALS AND METHODS

Materials (Berseem, spinach, raw mango and mint) required for RTS beverage preparation was bought from local market and were added according to Table 1 for preparing 1000ml beverage.

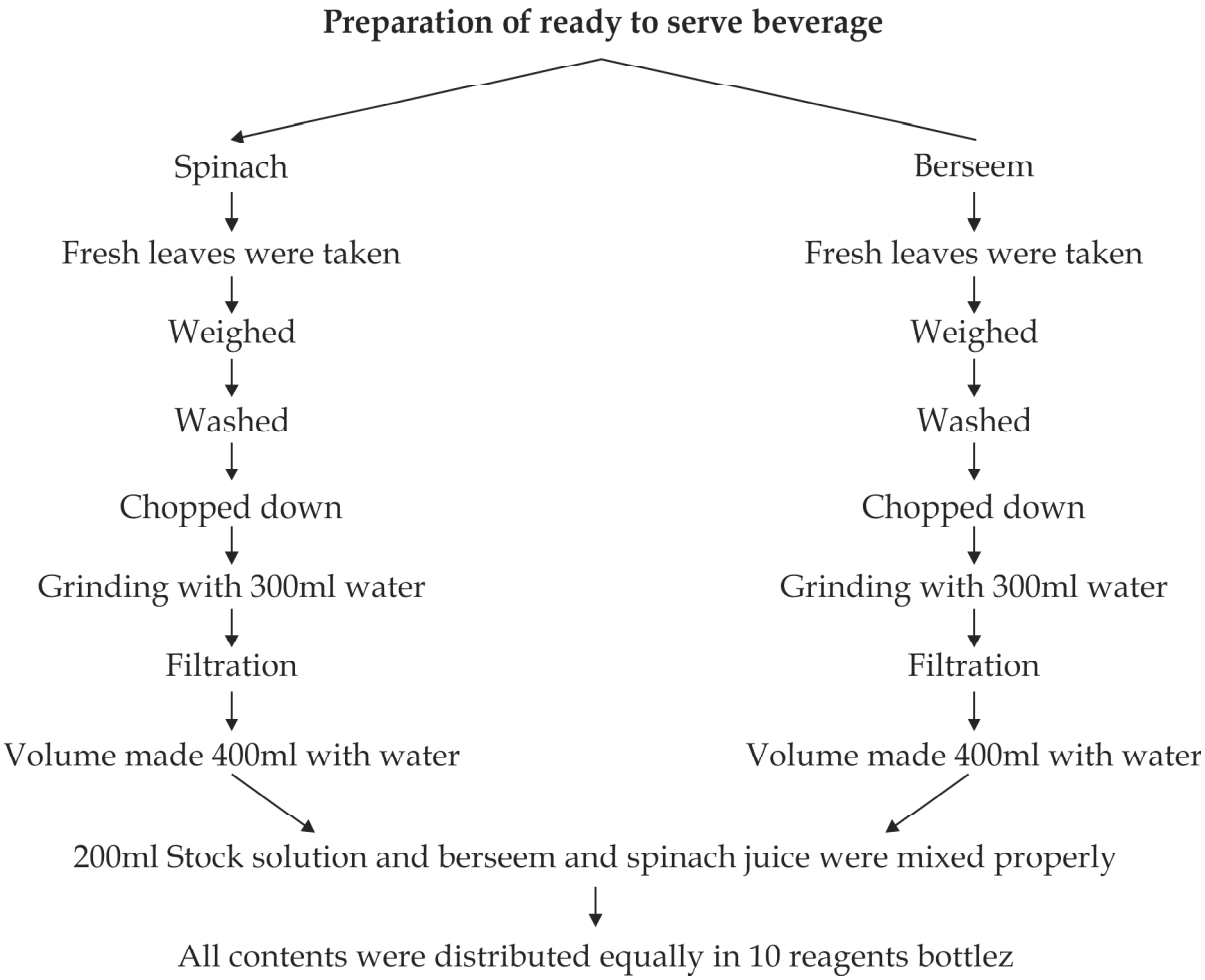

Figure 1: Preparation of ready to serve beverage

Table 1
Collection of raw materials for 1000ml RTS beverage:

	Items	*Amount for 1000ml RTS*
1.	Berseem	400ml
2.	Spinach	400ml 120g of each in water
3.	Mint	62.5g
4.	Tamarind	125g
5.	Raw mango	250g in 200ml of water
6.	Jaggery	250g
7.	Salt	25g
8.	Brown cumin seeds	25g

Preparation of RTS beverage

RTS beverage was prepared as shown in Figure 1.

Preparation of Stock Solution

Fresh mint, raw mango of uniform color, texture and firmness was selected for the experiment. Mint and mango were washed thoroughly in running water in order to remove dust and dirt particles and trimmed to remove undesirable portion with help of stainless steel knife. Mangoes and mint were then cut in small pieces before grinding.

Cumin seeds (heated over flame for 2 minutes), tamarind, Jaggery and salt were weighed on weighing balance according to the requirement. (Table 1). Weighed tamarind and jaggery were put in 200ml of hot water for few minutes, filtered via clean muslin cloth; mixed with chopped pieces of mango, mint together with cumin seeds and salt. Prepared mix was then put in grinder (Croma mixer grinder) to obtain the mashed product. After this, whole ingredients were again filtered via clean muslin cloth; the filtrate was kept in glass bottle named as stock solution.

Preliminary trials were conducted to prepare acceptable ready to serve beverage on the basis of sensory evaluation.

Processing and Storage Analysis

Ready to serve beverage was hot filled and capped in pre sterilized glass bottles in boiling water at 72°C for 2 minutes. Bottles were then thermal processed for 20 minutes followed by cooling to room temperature.

Ready to serve beverage (4° brix, pH 3.8) was stored at room (25 ± 5°C) and refrigeration temperature (3 ± 1°C) for 8 weeks. These samples were evaluated in triplicate for various physico chemical, microbiological and beverage making quality at an interval of every 15 days. The data obtained from physico chemical (AOAC 1984, Ranganna 2008), microbiological and sensory analysis (Larmond 1977) were analysed using ANOVA techniques.

RESULTS AND DISCUSSION

It was found that a ready to serve beverage can be prepared by adding 40 percent of berseem juice (1°brix, pH 6.18), 40 percent of spinach juice (1°brix, pH 6.17) and 20 percent of stock solution (25°brix, pH 3.42) with 70 ppm of sodium benzoate.

The ready to serve beverage (4° brix, pH 3.8) prepared from berseem and spinach (40 percent each) was subjected to sensory evaluation on 9 point hedonic scale.

Sensory analysis shows that 40:40 concentrations was more acceptable than other concentrations and then was assessed for physico- chemical and microbiological tests.

Table 2
Preparation of ready to serve beverage made from spinach and berseem from different concentrations

Contents	30:50	50:30	40:40	20:60	60:20
Berseem	30ml	50	40	20	60
Spinach	50	30	40	60	20
Jaggery	25g	25g	25g	25g	25g
Tamarind	12.5g	12.5g	12.5g	12.5g	12.5g
Raw mango	25g	25g	25g	25g	25g
Mint	6.25g	6.25g	6.25g	6.25g	6.25g
Cumin seeds	2.5g	2.5g	2.5g	2.5g	2.5g
Salt	2.5g	2.5g	2.5g	2.5g	2.5g
Na benzoate	0.3g	0.3g	0.3g	0.3g	0.3g

Composition of control (without berseem RTS) and RTS beverage with berseem

The composition of control (without berseem RTS) and RTS beverage obtained are given in Table 4

The results of storage analysis obtained are summarized as under:

1. The moisture, ash, protein and carbohydrate content in RTS beverage were more as compared to control sample which was without berseem.
2. There was no significant change in TSS, Vitamin C and Color of the RTS beverage stored either at room temperature (25 ± 5° C) and refrigeration (3 ± 1°C) temperatures at an interval of 0 day to 60 days.
3. During storage of RTS beverage there was significant increase in Titrable acidity and Reducing sugar, whereas significant decrease in pH and Total Sugar was observed.

Table 3
Sensory characteristics of ready to serve beverage made from spinach and berseem

	Sensory scores						
Ratios of B:S	*Appearance*	*Taste*	*Color*	*Flavor*	*Smell*	*Overall acceptability*	*Mean*
30:50	6	6	5	5	7	7	5.9
50:30	7	5	6	7	8	8	6.4
40:40	7	8	6	7	8	8.1	7.8
20:60	5	4	5	4	7	6	5.6
60:20	6	5	6	6	7	7	6
F value	*	*	*	*	*	*	

F value (5%) F, 0.05,4,84 = 2.48, since test statistics is much larger than critical value, we reject null hypothesis of equal populations means and conclude that there is statistically significant difference among population means.
* = significant
ns = Non significant
B = Berseem
S = Spinach

Table 4
Composition of RTS beverage without berseem (Control) and with berseem

Parameters	*Composition**	
	Sc Control (without berseem)	*Sb RTS with Berseem*
Total soluble		
Solids (°Brix)	22	18.4
Total solids %	21.85	30.29
pH	3.42	3.51
Acidity % citric acid	0.224	0.332
Color at 440nm	0.308	0.379
Moisture %	78	69.71
Proteins %	4.55	9.7
Ash %	1.98	2.77
Carbohydrate %	13.6	15.5
Reducing sugar %	4.9	1.9
Ascorbic acid mg/100ml	0.504	0.45
Fat	1.87	2.32
Iron (mg/20g)	4.76	5.24
Calcium (mg/100g)	80.5	106
Plating LB		
10^1	1×10^1	1×10^1
10^3	2×10^3	2×10^2
10^5	3×10^5	3×10^5
YPD		
10^1	Nil	Nil
10^3	Nil	Nil
10^5	Nil	Nil

*On day 0 or on fresh weight basis

The RTS beverage quality reduced significantly during storage beyond 30 days. However, the reduction in sensory scores was higher when samples were stored at room temperature.

There was no microbe growth in any of the RTS beverage, when the sample was plated on media (for Total Plate count, Yeast and Mold Count and Enterobacteria) in triplicate. However very few colonies were started occurring after 15 days.

On the basis of above findings, it may be concluded that 40: 40 concentration of berseem and spinach was most suitable for the preparation of RTS beverage and can be stored satisfactorily for a period of 4 weeks at refrigeration temperature (5 ± 1°C) with addition of sodium benzoate. Further RTS beverage which was stored at refrigeration temperature was more acceptable than beverage stored at room temperature (25 ± 5° C).

Table 5
Changes in color of RTS beverage during storage at room temperature (25 ± 5° C) and refrigeration (3 ± 1°C) temperature

Storage Temp. (S)	*Color (nm)*				
	Storage period days (SS)				
	0	*15*	*30*	*45*	*60*
Room temperature (25 ± 5° C)	0.370	0.368	0.366	0.366	0.366
Refrigeration (3 ± 1°C)	0.379	0.377	0.375	0.373	0.370

F Value (5%) *ns* at room and refrigeration temperature
*Significant
Ns Non significant

Table 6
Changes in total soluble solids (Brix) of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature.

Storage Temp. (S)	*Total soluble solids*				
	Storage period days (SS)				
	0	*15*	*30*	*45*	*60*
Room temperature (30 ± 5° C)	20.2	19.8	19.6	19.3	18.9
Refrigeration (5 ± 1°C)	19	18.7	18.7	18.6	18.6

F Value (5%) *ns* at room and refrigeration temperature
Since test statistic is same as the critical value, we will not reject the null hypothesis of equal population means and conclude that there is a (no statistically) significant difference among the population means.
*Significant
ns Non significant

Titrable Acidity

Table 7
Changes in titrable acidity (%) of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature.

Storage Temp. (S)	*Titrable acidity*				
	Storage period days (SS)				
	0	15	30	45	60
Room temperature (30 ± 5° C)	0.41	0.45	0.48	0.52	0.60
Refrigeration (5 ± 1°C)	0.38	0.43	0.46	0.51	0.59

F Value (5%) ns at both room and refrigeration temperature
*Significant
ns Non significant

pH

Table 8
Changes in pH of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature.

Storage Temp. (S)	pH				
	Storage period days (SS)				
	0	15	30	45	60
Room temperature (30 ± 5° C)	3.51	3.49	3.48	3.3	3.2
Refrigeration (5 ± 1°C)	3.65	3.59	3.52	3.47	3.4

F Value * at refrigeration temperature and ns at room temperature
*Significant
ns Non significant
Reducing sugars

Table 9
Changes in reducing sugars (%) of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature.

Storage Temp. (S)	*Reducing sugars*				
	Storage period days (SS)				
	0	*15*	*30*	*45*	*60*
Room temperature (30 ± 5° C)	1.22	1.29	1.55	1.78	1.93
Refrigeration (5 ± 1°C)	1.9	1.94	1.97	2.2	2.50

F Value (5%) * at room and refrigeration temperature
*Significant
ns Non significant

Ascorbic Acid

Table 10
Changes in ascorbic acid (mg/100 ml) of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature

Storage Temp. (S)	*Ascorbic acid*				
	Storage period days (SS)				
	0	*15*	*30*	*45*	*60*
Room temperature (30 ± 5° C)	3.52	3.50	3.49	3.48	3.44
Refrigeration (5 ± 1°C)	3.45	3.42	3.40	3.40	3.37

F Value (5%) *ns* at refrigeration and room temperature
*Significant
ns Non significant
Sensory quality

Table 11
Overall acceptability of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature

Storage Temp. (S)	*Overall acceptability*				
	Storage period days (SS)				
	0	*15*	*30*	*45*	*60*
Room temperature (30 ± 5° C)	8.1	7.8	7.4	6.5	5
Refrigeration (5 ± 1°C)	8.1	7.9	7.6	7	6

F Value (5%) *ns* at refrigeration and significant at room temperature
*Significant
ns Non significant

MICROBIOLOGICAL ANALYSIS

Total Plate Count

Table 12
Change in total plate count (CFU/ml) of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature.

Storage Temp. (S)	*Change in total plate count (CFU/ml)* *Storage period days (SS)*				
	0	*15*	*30*	*45*	*60*
Room temperature (30 ± 5° C)	2.1×10^3	3×10^3	4.4×10^3	7.6×10^3	1.8×10^4
Refrigeration (5 ± 1°C)	2.1×10^3	2.7×10^3	3.3×10^3	0.5×10^4	0.16×10^5

F Value * values were significant at room and refrigeration temperature
*Significant
ns Non significant

Yeast and Mold Count

Table 13
Change in Yeast and mold count (CFU/ml) of RTS beverage during storage at room temperature (30 ± 5° C) and refrigeration (5 ± 1°C) temperature

Storage Temp. (S)	*Yeast and mold count (CFU/ml)*				
	Storage period days (SS)				
	0	15	30	45	60
Room temperature (30 ± 5° C)	0	0	0	2×10^2	5×10^3
Refrigeration (5 ± 1°C)	0	0	0	0	1×10^2

F Value
*Significant

REFERENCES

AOAC, (1984), Official Methods of Analysis (16th Edn.), Association of Official Analytical Chemists, Washington, DC.

Dixit O.P, Singh U.P. and Gupta J.N. (2008), Significance of pollination in seed setting efficiency of Berseem (Trifolium alexandrinum L). j 1439-037x. 1989. tb00693. x.

Larmond E, (1977), Laboratory methods of sensory evaluation of foods. Publication 1963, Canada Deptt of Agric., Ottawa.

Malaviya et al (2010). Crop Profile – BERSEEM, ICAR.

Ranganna S, (2008), Handbook of Analysis and Quality Control for Fruits and Vegetable Products. Tata MaGraw Hill publishing company limited, New Delhi.

❍❍❍