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Physico: Chemical analysis of Probiotic/Synbiotic whey drink with orange juice

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Abstract
The present investigation was carried out for formulation of functional Probiotic/Synbiotic whey drink with orange juice and also to study their physico-chemical analysis. Whey based functional drinks were in two different forms; A [whey + sugar @ 10 % (w/v) + orange juice@ 10 % (v/v)], B [A + inulin@ 3 % (w/v)], and inoculated with probiotic culture *Lb. rhamnosus* @ 2.0% v/v. The two blends (A and B) developed were subjected to chemical analyses initially for the fresh (0 day) and up to 28 days of stipulated refrigerated storage at an interval of 7 days at 4 ± 1ºC. The average chemical composition (% w/w) for the whey used to manufacture whey drink was moisture 93.56, T.S.; 6.4, fat 0.50; protein, 0.7; ash, 0.40 and carbohydrate (by difference), 4.80. In case of blends A and B, the T.S. increased to (A) 9.38 and (B) 12.11. The increase in protein, carbohydrates content of the blends (A and B) was 0.21 to 0.27; 9.39 to 12.17 respectively. during all the periods (0 to 28 days); the changes were found statistically non-significant, Similarly the increase in tyrosine content (0.51 to 0.67 µg/5ml) for the treatments (A and B) was relatively very low during entire length of storage period.

Keywords: Synbiotic whey drink, Probiotic culture *Lb. rhamnosus*, Orange juice, Inulin, Storage study, Chemical analysis

Introduction
The health giving and vitalizing properties of fermented milk / milk like beverage apart from its nutritive value have been documented since ancient times. Some of the reported nutritional and physiological benefits of fermented milks are the promotion of growth and digestion, settling effect on gastrointestinal tract (GIT) by decreasing harmful bacteria, improving bowel movement, ameliorating immunity and mineral absorption, suppression of cancer and lowering of blood cholesterol (Welch, 1987; Yukuchi *et al.*, 1992; Butriss, 1997) [12, 13, 2]. Since whey has a number of nutritional aspects and to increase the nutritional value of fermented as well as non fermented whey drinks have emerged in the market. Some of the arguments available to a marketing man for whey drinks are also very powerful if correctly handled as follows: whey is a genuine thirst quencher; whey drinks are light and refreshing but less acidic than unlike most soft drinks, there are health and nutritional arguments, and there are good potential profit margins.

A whey drink can replace much of the lost organic and inorganic to the extracellular fluid. Whey, which is so rapidly assimilable, forms an ideal metabolic substrate. L (+) Lactic acid culturing the whey during production of a drink increases the level of metabolically active L (+) lactic acid. The utilization of whey as a drink has a two-fold advantage: Large volumes of whey are used direct from the cheese vat, eliminating disposal problems. There are simple processing and common equipment requirements. German market emphasizes the word 'Molke', or whey and has many whey drinks in market (Prendergast, 1985) [11].

Whey: A vehicle for Probiotics
Lactobacilli and bifidobacteria must have the ability to survive the harsh conditions in the gut if they are to be used as dietary adjuncts in fermented foods. Proteolytic enzyme *i.e.* pepsin can hydrolyze the proteins of the outer layer of bacterial cells in acidic condition (optimum pH 1.0-2.5) in the stomach. Moreover, bacteria have cell membranes consisting of lipids and fatty acids that are very susceptible to destruction by bile salts. However, the survival during passage through the GI tract is influenced by the nature of food carrier used for the delivery of probiotic. Whey can protect the cell from reaching the death by increasing the overall pH and inhibiting digestive protease activity (Charteris *et al*., 1999) [3].

Probiotics such as *Lactobacillus* spp. and *Bifidobacterium* spp. can be used in formulation of probiotic/synbiotic whey drinks to further improve the nutritional value with better texture and appealing product.

Objectives

1. To formulate functional synbiotic whey drink containing probiotic culture (*Lb. rhamnosus MTCC 5462* previously known as *Lb. acidophilus* V3) and Prebiotic (Inulin) and also to analyze the gross chemical composition (fresh product), biochemical changes (extent of proteolysis, lipolysis and acidity development) of fresh product as well of stored product.
2. To check the acceptability of the fresh as well as stored product at the regular interval of 7 days till 28th days of the refrigerated storage (4 ±1 °C).
3. To check effect of some food ingredient (sugar)/additives (orange juice) on the growth of Probiotic culture during storage.
4. To check the shelf life of the product using refrigerated storage study determined at refrigerated temperature of 4 ±1 ºC.

Materials and Methods

Preparation of synbiotic whey drink

The work was carried out in the Department of Dairy Microbiology SMC College of Dairy Science, Anand. The raw materials, which were used during the course of study along with their sources, are delineated here under.

The fresh cow milk was collected from Livestock Research Station, Anand for manufacturing of synbiotic whey drink. Inulin was added @ 3 % (w/v) for the formulation of the final product. Oraftii Ltd, Belgium, supplied the inulin having trade name Raftiline. Sugar For fortification of synbiotic whey drink with sweetener high quality sugar, free from an impurity was purchased from local supplier.

Probiotic culture and its maintenance

The culture used in the present study was *Lb. rhamnosus MTCC 5462* (previously known as *Lb. acidophilus V3*) obtained from the Culture Collection of Dairy Microbiology Department, SMC College of Dairy Science, Anand. The culture @ 2 % v/v was propagated in sterilized skim milk (10 % T.S., Sagar skim milk powder, Dudhsagar) for 16 h and in sterile whey for 12 h and stored at 5 ± 2 °C. The transfer was given every week during the course of the study.

Preparation of symbiotic whey drink

A [whey + sugar @ 10 % (w/v) + orange juice@ 10 % (v/v)]

B [A + inulin@ 3 % (w/v)], and inoculated with probiotic culture *Lb. rhamnosus* @ 2.0% v/v.

Receiving raw cow milk and then Filtered through muslin cloth, the raw milk is Heating to 95° ºC for 5 min, then immediately Cooling up to 70°C, Addition of citric acid (1.5% solution) and Settling (left undisturbed for 10min) to obtain Paneer or coagulated mass then separation of Whey Boiling up to 100 ºC to obtain clear whey, immediately Cooling is done at 37 ºC. Filter the whey and to that Addition of ingredients sugar @ 10% (w/v) for plain whey drink (Blend A), whey drink with Inulin @ 3% (w/v) (Blend B),both the samples are Heat treatment up to 95 °C for 5 min, Cooling is to be done at 37 °C for both the samples. Inoculation of culture (*Lb. acidophilus* V_3, @ 2 % v/v), Incubation (37 °C) till acidity reaches 0.7 % L. A, Shifting in refrigerator and Cooling at (4 ± 1 °C) and then Addition of orange fruit juice (@ 10% v/v. finally prepared fermented whey is filling in glass bottles (200 ml) Crown capping and kept in Refrigerated storage of whey drink (Blend A & B) at 4 ± 1 °C.

Chemical analysis of whey drinks

The whey as well as whey drinks (A and B) was analysed for the chemical attributes *viz.* pH, acidity, moisture, fat, protein, total solids, ash contents, carbohydrates (by difference), free fatty acids, and tyrosine value were estimated during 0 day. The inoculated whey (A and B) before incubation for making whey drinks were analysed for pH and acidity. The final product (whey drink A and B) were analysed in duplicate for the following chemical attributes: pH, acidity, free fatty acids, and tyrosine value at 0, 7, 14, 21 and 28 days intervals during refrigerated storage.

Determination of pH

Electronic pH meter used to determine pH of cow milk as well as whey (Model CYBERSCAN 2100 manufactured by EUTECH Instruments, Singapore).

Determination of titratable acidity

Titratable acidity of whey drinks was estimated by the procedure described in ISI (1981) [10].

Estimation of fat content

Fat content in whey drink samples was determined in duplicate as per the procedure given in ISI (1981) [10] by Mojonnier method.

Estimation of Total Solids and Moisture

Total solids (T.S.) in whey drink samples were determined according to the procedure described in IS: 1479 (II) (1961).

Estimation of Ash Content

As per procedure given in ISI (1981) [10].

Estimation of Total Protein

Protein content of whey drink samples in duplicate was estimated by Kjeldahl method as described in IS: 9617 (1980) [9].

Estimation of Free Fatty Acids (FFA)

The free fatty acids content in whey drink samples was determined by the method described by Deeth and Fitz-Gerald (1976) [4].

Determination of Tyrosine Value

The tyrosine value was determined by modified Hull method (Hull, 1947) [7].

Results and Discussions

Acidity

The influence of fortification of additives/ingredients on the lactic acid content of synbiotic whey drink (A and B) during refrigerated storage (4 ± 1 °C) are shown in figure1.a. The table -1.a and table 1.b - indicates mean values and ANOVA table, respectively. It indicates lactic acid content for the treatments A and B were non- significant. Initial development of acidity at 0 day was 0.72, 0.70 (% LA) for treatment A and B, respectively, which was statistically non- significant.

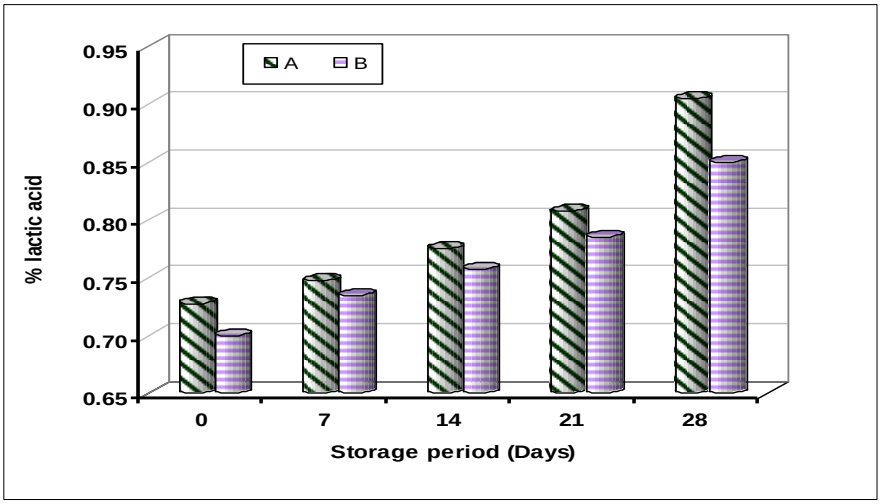

Fig 1.a: Changes in lactic acid contents of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1°C).

It was observed that for both treatments during storage period (0 to 28 days), the acidity increased at relatively faster rate (0.70 to 0.90 % L.A.). And they found significant. ($p< 0.01$) for both the samples. The changes in lactic acid contents were initially slow (up to 21 days), but there was remarkable increase during the last interval of storage (0.78 to 0.90 % L. A.). The increased acidity it may be due to supplementations of Orange juice.

Table-1.a: Changes in lactic acid contents of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Storage period (Days)	0	7	14	21	28	Treatment mean
A	0.7275	0.7475	0.7750	0.8075	0.9050	0.7925
B	0.7000	0.7350	0.7575	0.7850	0.8500	0.7655
Period mean	0.7138	0.7413	0.7663	0.7963	0.8500	
Total: 31.160	General mean: 0.779					

CD value 0.053, highly significant (P<0.01), NS- Non significant.

Table 1.b: ANOVA table for lactic acidity of functional probiotic/symbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Source	DF	SS	MS	F Cal	F Tab (1%)	F Tab (5%)	S Em	CD	Test
Treatment	1	0.007	0.007	2.664	7.560	4.170	0.012	-	NS
Interval	4	0.127	0.032	11.580	4.020	2.690	0.018	0.053	**
T x I	4	0.002	0.001	0.202	4.020	2.690	0.026	-	NS
Error	30	0.082	0.003	CV %: 6.715					

** Highly significant (P<0.01), NS- Non significant, DF-Deviation factor, SS-Sum of square, MS-Mean square, SEm-standard error mean.

Gandhi (1989) [6] patented acido whey-lactic fermented noncarbonated beverage produced from cheese whey or paneer whey, heated 85 °C to 90 °C / 20 min and cooled to 40 °C inoculated with *Lb. acidophilus* culture (@ 2%). The final product had 0.8 to 0.85 % (LA) acidity.

pH Values

The changes in pH values due to fortification of ingredients in synbiotic whey drink (A and B) during refrigerated storage (4 ± 1 °C) are shown in figure- 2.a. The table- 2.a indicates mean values. The initial pH value at 0 day was almost similar (around 3.9) as seen in table 2.b for samples A and B. The treatments A and B were statistically non significant at 0 day storage period.

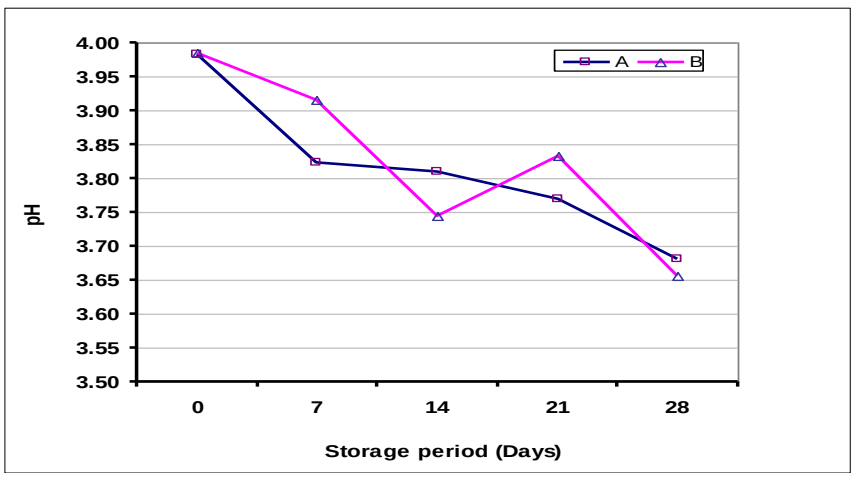

Fig 2.a: Changes in pH values of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

For samples A and B nearly identical trend was observed for decline in pH from 3.98 to 3.68 (Fig 2.a & Table: 2.a) for the entire refrigerated period of storage from 0 to 28 days. It was observed that at the end of storage period of 28 days, sample A, B were found statistically non-significant.

Table 2.a: Changes in pH values of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Storage period (Days)	0	7	14	21	28	Treatment mean
A	3.9825	3.8225	3.8100	3.7700	3.6800	3.8130
B	3.9850	3.9150	3.7450	3.8325	3.6550	3.8265
Period mean	3.9838	3.8688	3.7775	3.8012	3.6675	
Total:152.79	General mean: 3.819					

CD value 0.108, highly significant (P<0.01), NS- Non significant.

Table 2.b: ANOVA table for pH of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Source	DF	SS	MS	F Cal	F Tab (1%)	F Tab (5%)	S Em	CD	Test
Treatment	1	0.002	0.002	0.164	7.560	4.170	0.024	-	NS
Interval	4	0.437	0.109	9.815	4.020	2.690	0.037	0.108	**
T x I	4	0.033	0.008	9.815	4.020	2.690	0.053	-	NS
Error	30	0.334	0.011	CV%: 2.76					

**Highly significant (P<0.01), NS- Non significant, DF-Deviation factor, SS-Sum of square, MS-Mean square, SEm-standard error mean.

However, during storage from 0 to 21 days a different trend of reduction in pH was observed for the sample B, which was also statistically significant (P<0.05). At the end of refrigerated storage (28 days), both samples showed significant drop in pH value in comparison to fresh samples.

Beucler and co workers (2005) [1] developed beverage from whey permeate, Beverages were designed to be similar in all aspects to commercial beverages; therefore pH was measured across all beverages, commercial and Whey permeate. The average pH of the commercial beverages was 3.69 ± 1.27. The pH of commercial beverages ranged from 2.78 ± 0.01 to 6.69 ± 0.00. Most commercial beverages (14/15) were between 2.78 ± 0.01 to 4.15 ± 0.02. The pH of the Whey Pearmeate beverages increased as more permeate was added to each beverage. The control formulations made with 0% Whey permeate had an average pH of 3.03 ± 0.08 whereas the beverage formulations containing 100% permeate had an average pH of 4.37 ± 0.015. Hydrolysis had no effect on pH.

Djuric and co workers (2004) [5] worked on Development of whey based beverage consisting of whey, fruit components (orange, pear, peach, and apple), citric acid and sucrose. The pH changed within a narrow range. The peach–whey beverage containing 6% of dry matter and 2% of sucrose as well as having pH 3.6 proved to be the best. In the present investigation too the pH recorded was similar.

Free fatty acid contents

The influence of addition of additives / ingredients on the free fatty acid content of control whey drink as well as synbiotic whey drink (A and B) during refrigerated storage (4 ± 1 °C) have been shown in figure- 3.a. The mean values for treatments and ANOVA for the same are shown in table - 3.a and 3.b respectively. When treatments were subjected to statistical analysis, it was observed that for both the treatments (A and B) individually as well as at each and every period intervals, ranging from 0 to 28 days, the change in free fatty acid contents were non-significant. In the fresh whey drink samples at 0 day, the FFA value was around 0.55 µg/ml for both the samples.

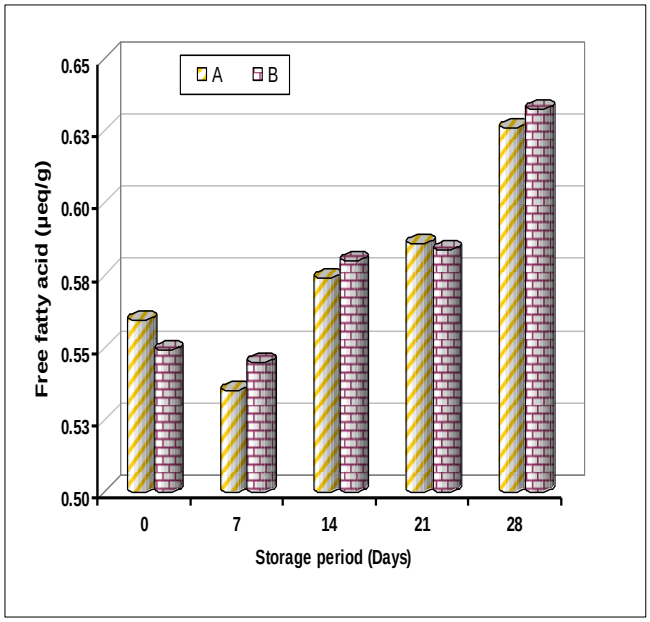

Fig 3.a: Changes in free fatty acid contents of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

The end of 28 days storage period the FFA value increased around 0.63 µg/ml for both the samples. The interaction (T x I) between treatments (T) and the storage period (I) was also non- significant. The main reason for apparently no changes in FFA content during storage can be contributed to very little fat (0.32 - 0.34%) in whey drinks and consequently no lipolytic changes.

Table 3.a: Changes in free fatty acid contents of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Storage period (Days)	0	7	14	21	28	Treatment mean
A	0.5595	0.5348	0.5738	0.5858	0.6258	0.5759
B	0.5490	0.5448	0.5800	0.5835	0.6325	0.5780
Period mean	0.5543	0.5398	0.5769	0.5846	0.6291	
Total - 23.077	General mean: 0.577					

Table 3.b: ANOVA table for free fatty acid contents of functional probiotic/ synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 ºC).

Source	DF	SS	MS	F Cal	F Tab (1%)	F Tab (5%)	S Em	CD	Test
Treatment	1	0.000	0.000	0.011	7.560	4.170	0.014	NS	NS
Interval	4	0.037	0.009	2.429	4.020	2.690	0.022	NS	NS
T x I	4	0.001	0.000	0.036	4.020	2.690	0.031	NS	NS
Error	30	0.116	0.004	CV%: 10.76					

NS- Non significant, DF-Deviation factor, SS-Sum of square, MS-Mean square, SEm-standard error mean.

Tyrosine Contents

The changes in tyrosine content of control whey drink as well as synbiotic whey drink (A and B) during refrigerated storage (4 ± 1 °C) are shown in figure- 4.a. The mean values and ANOVA for the treatments are shown in table-4.a & 4.b and The variations in tyrosine content (0.51 to 0.67 µg/5ml) for the two treatments were relatively very low during entire length of storage period from 0 to 28 days as shown in table.

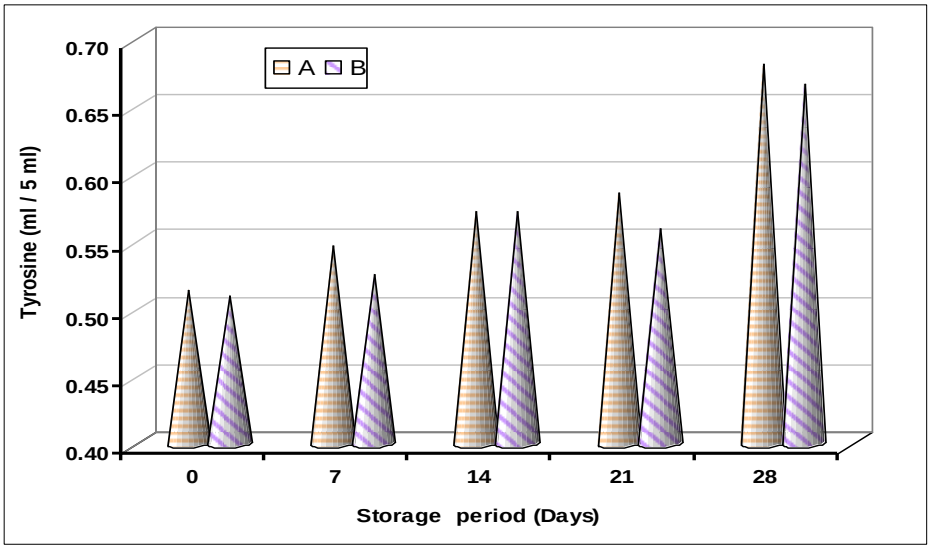

Fig 4.a: Changes in tyrosine contents of functional probiotics/ synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

For the fresh samples (0 days) the tyrosine values varied marginally (around 0.51 µg/5ml). At the end of 28 days storage, while it increased from 0.51 to 0.67mg/ 5ml. It was observed from the data that there was very slight increase in tyrosine value during 0 to 28 days of storage periods in both the samples, indicating low degree of proteolysis in samples.

Table 4.a: Changes in tyrosine contents of functional probiotic /synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Storage period (Days)	0	7	14	21	28	Treatment mean
A	0.5145	0.5463	0.5718	0.5858	0.6810	0.5799
B	0.5100	0.5260	0.5720	0.5593	0.6665	0.5668
Period mean	0.5123	0.5364	0.5719	0.5725	0.6738	
Total:23.077	General mean: 0.577					

Table 4.b: ANOVA table for tyrosine contents of functional probiotic/synbiotic whey drink with orange juice during refrigerated storage (4 ± 1 °C).

Source	DF	SS	MS	F Cal	F Tab (1%)	F Tab (5%)	S Em	CD	Test
Treatment	1	0.002	0.002	0.537	7.560	4.170	0.013	NS	NS
Interval	4	0.121	0.030	9.355	4.020	2.690	0.020	0.058	**
T x I	4	0.001	0.000	0.076	4.020	2.690	0.028	NS	NS
Error	30	0.097	0.003	CV %: 9.94					

**Highly Significant (P<0.01), NS-Non significant, DF-Deviation factor, SS-Sum of square, MS-Mean square, SEm-standard error mean.

When treatments were subjected to statistical analysis, it was seen that among two treatments, there was non-significant relationship. However, when storage periods were compared, the values were found highly significant, especially for fresh (0 days) and 28 days refrigerated stored whey drink samples. It was also observed that the interaction among the treatments and periods (T x I) were non-significant.

Conclusion

These products can surge ahead in market as appealing functional whey drink for consumers as an alternative of carbonated soft drinks and give health benefits due to presence of probiotic culture, inulin, whey proteins & other whey constituents as well as orange fruit juice.

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