The Pharma Innovation Journal 2022; SP-11(7): 4795-4800

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277-7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2022; SP-11(7): 4795-4800

www.thepharmajournal.com
Received: 15-05-2022
Accepted: 20-06-2022

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# Impact of refrigerated storage on sensory and microbiological quality parameters of resveratrol fortified yoghurt

**Harshita Sonarthi, Sivakumar S, Rekha Chawla and Veena N**

**Abstract**
Yoghurt, the most popular fermented dairy product blended with functional ingredients like whey protein isolate and sodium caseinate based resveratrol encapsulated powder was the target of study to provide health benefits to the consumers. To evaluate the shelf life of product functional yoghurt the product was stored at refrigeration (4±1 ºC) temperature till its spoilage. The product was analysed for its sensory attributes and microbiological parameter at the regular interval of 3 days at refrigeration (4±1 ºC) temperature conditions. During storage all the sensory attributes viz., colour and appearance, texture, flavor, and overall acceptability decreased in both control and experimental samples at refrigeration temperature. The microbiological analysis of the yoghurt samples during storage revealed that control yoghurt and experimental sample was acceptable up to 15 days. After this period, coliform and yeast & mould counts were increased beyond the prescribed limit of FSSAI standards. The microbial counts correlated with the sensory scores of the product.

**Keywords:** Resveratrol, yoghurt, encapsulation, sensory, microbiology, refrigerated storage

## Introduction
Milk is a complex mixture of proteins, fat, lactose and minerals play a crucial role in flavour and textural properties of milk products. Milk fermentation is one of the oldest methods practiced by the human beings to preserve milk with an extended shelf life. Yoghurt is one of the most popular fermented dairy products which have great consumer acceptability due to its health benefits other than its basic nutrition. Yoghurt contains lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Weerathilake *et al*., 2014) [1]. Resveratrol is a naturally occurring polyphenol, found in grapes, grape products such as wine, as well as other botanical sources such as peanuts (Bradamante *et al*., 2004) [2]. It is well known for its multiple pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, anticancer, neuroprotective and cardioprotective effects. *In vitro* evidence of resveratrol efficacy is widespread, however, many concerns regarding its effectiveness *in vivo* arise from its poor stability in vitro and bioavailability following oral ingestion (Francioso *et al*., 2014) [3]. It is well known that phenolic compounds interact with other macromolecules during the digestive process, such as fibers, lipids, proteins and food polysaccharides which affect their bioaccessibility. Encapsulation, ensures the coating of the active component and its targeted delivery to a specific part of the digestive tract and controlled release (Grgić, *et al*., 2020) [4]. Spray-drying is used for the production of powders containing the encapsulated material as tiny droplets enveloped in a polymer wall matrix (Gharsallaoui *et al*., 2007) [5]. The sensory properties of yoghurt gels are highly influenced by the protein content and the total solids content of the yoghurt milk. The refrigerated storage plays an important role in sensory and microbiological parameters of the yoghurt. Keeping in view of the all the facts, present study was focused on to study the effect of refrigerated storage on the sensory and microbiological changes in resveratrol fortified yoghurt.

## Materials and Methods
**Raw materials:** Fresh, clean and good quality mixed (cow and buffalo) raw milk was obtained from Experimental Dairy Plant of College of Dairy Science and Technology. Materials required for encapsulation are Whey protein isolate, Sodium caseinate, Resveratrol, Olive oil, Maltodextrin, etc. were purchased from the reputed firms for preparation of encapsulated resveratrol powder. Yoghurt starter culture (*L. bulgaricus* and *S. thermophilus*) was procured

from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal, India.

**Preparation of yoghurt and its quality evaluation during storage**

Standardized milk (Fat 3%, SNF 10%) obtained was converted to yoghurt using method described by (Tamime and Robinson, 1999) [6] along with addition of 1% whey protein isolate and sodium caseinate based encapsulated resveratrol powder was offered to semi trained panel of judges for sensory evaluation on 9 point hedonic scale (Lawless and Heymann, 1998) [7]. The optimised yoghurts were packed in polystyrene cups and stored at 5 ºC for 15 days and the product was analysed for its changes in sensory parameters and microbiological at 3 days interval. Lactic acid bacteria, yeast & mold and coliform count were recorded as per procedure by mentioned by (Wehr and Frank, 2004) [8].

**Statistical analysis**

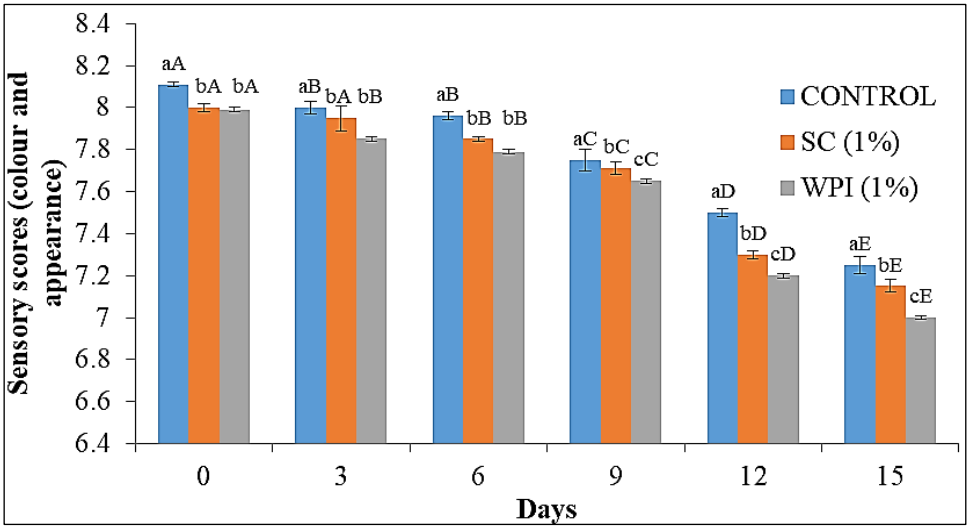
The data of the samples obtained from various experiments were statistically analyzed by Analysis of Variance using statistical software SPSS 20.0 (IBM).

## Results and Discussion

**Changes in sensory evaluation of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage**

**Colour and Appearance**

The colour and appearance scores presented in Figure 1 shows that the fresh control yoghurt scored 8.11, while SC based fortified yoghurt scored 8.00 and WPI based fortified yoghurt scored 7.99. For 0 and 3rd day the scores for SC based fortified yoghurt 1% shows non-significant difference ($p \leq 0.05$) after that the scores reduced significantly because of slight expulsion of whey. For WPI based fortified yoghurt 1% the scores are decreased but showed no significant difference was observed on 3rd and 6th day after that the scores were reduced significantly. The scores were decreased because of the incorporation of encapsulated resveratrol powder and syneresis. At the end of 15th day control yoghurt scored 7.25, while SC based fortified yoghurt scored 7.15 and WPI based fortified yoghurt scored 6.99. WPI got lowest score because of it colour. The judges score was less due to development of dull appearance.




Average value n=6

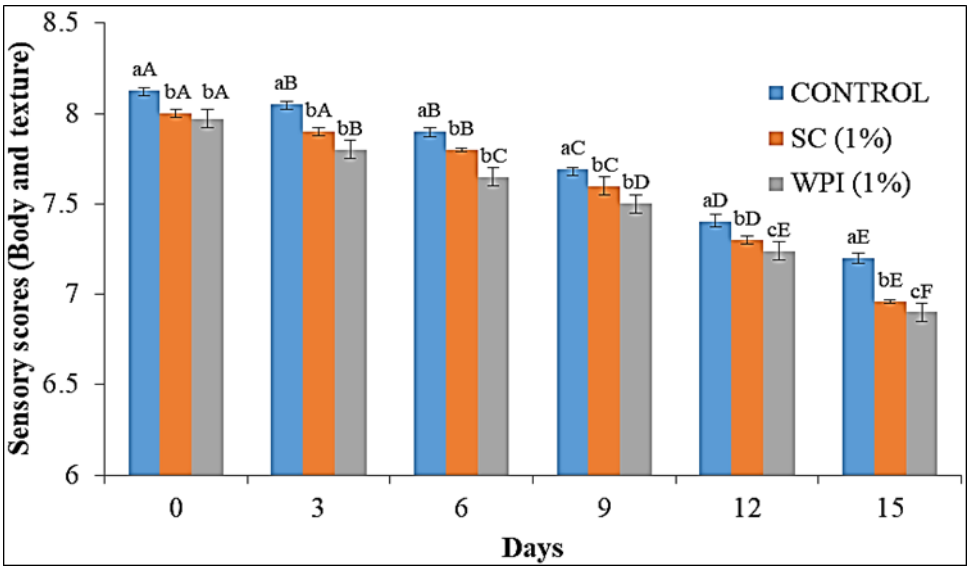
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean ± SE). Small letter shows difference between the samples while capital letter shows difference between the days.

**Fig 1:** Changes in colour and appearance of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

During storage, the appearance of yoghurt was affected and not acceptable which ultimately deteriorated the quality of yoghurt. It may be due to the growth of some yeast and mould in yoghurt during storage (Tarakci and Kucukoner, 2003) [9]. The effect of storage between the samples (control and experimental yoghurt) was found significant for colour and appearance at ($P \leq 0.05$).

**Body and Texture**

The body and texture scores presented in Figure 2 shows that the fresh control yoghurt scored 8.13, while SC based fortified yoghurt scored 8.04 and WPI based fortified yoghurt scored 7.96. SC based fortified yoghurt 1% shows nonsignificant difference ($p \leq 0.05$) during 0 and 3rd day but after that the scores were declined significantly. This is because at the starting the gel formation and bonding was high, but as the days increased because of the microbial action the gel loses its strength and showed loose body and syneresis. The optimized product (WPI/SC) showed significant difference($p \leq 0.05$) on 12th and 15th day.




Average value n=6

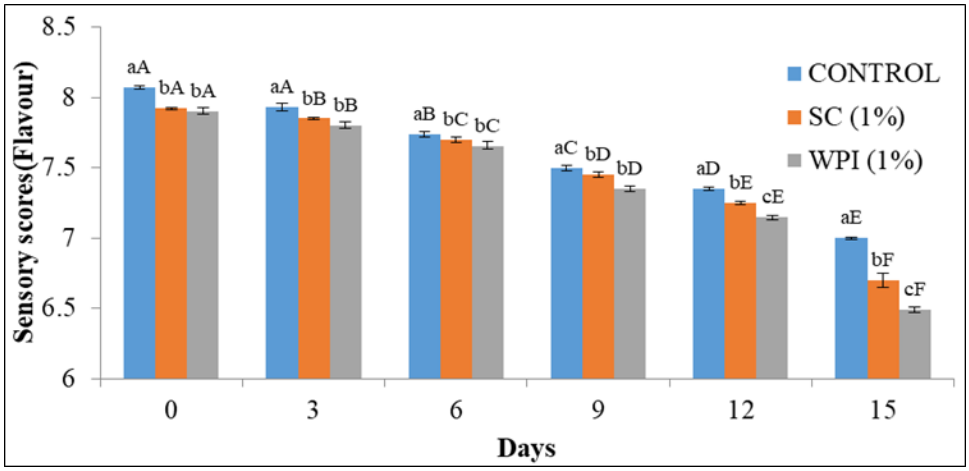
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean±SE). Small letter shows difference between the samples while capital letter shows difference between the days.

**Fig 2:** Changes in body and texture of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

At the end of 15th day control yoghurt scored 7.2, while SC based fortified yoghurt scored 6.96 and WPI based fortified yoghurt scored 6.9. This may be because of almost same protein content of SC and WPI. Over all, during storage the scores for body and texture are decreased significantly between storage period and samples.

**Flavour**

The flavour scores presented in Figure 3 shows that the fresh control yoghurt scored 8.07, while SC based fortified yoghurt scored 7.92 and WPI based fortified yoghurt scored 7.91. (WPI 1%) showed significant difference with control this may be because of release of some resveratrol content. At the end of 15th day control yoghurt scored 7.0, while SC based fortified yoghurt scored 6.60 and WPI based fortified yoghurt scored 6.49. Finally, at the end of the storage period the scores for the flavour of the control, SC based fortified yoghurt (1%) and WPI based fortified yoghurt (1%) decreased significantly($p \leq 0.05$), this might be because of some amount of resveratrol may be released from encapsulated substances and also may be due to microbial effect. Abrahamsen (1978) [10] reported that the decrease in flavour was due to the proteolytic activity of bacteria and the production of higher acidity in yoghurt. Loss of flavour can be attributed to fat and protein degradation (Mottar *et al*., 1989) [11].




Average value n=6

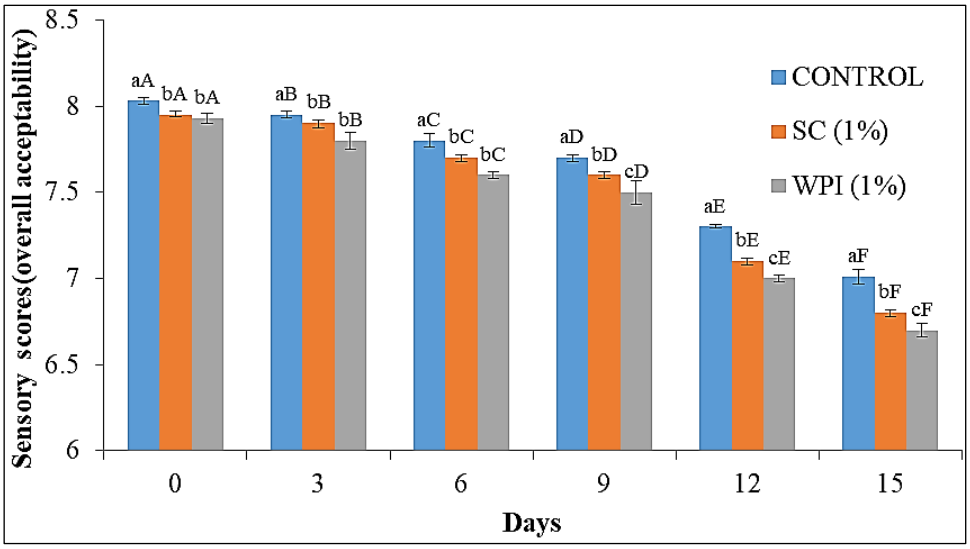
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean ± SE). Small letter shows difference between the samples while capital letter shows difference between the days.

**Fig 3:** Changes in flavour of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

**Overall acceptability**

During the storage period the overall acceptability of the samples decreases significantly ($p \leq 0.05$) because of syneresis, bitter flavour and loose body. The scores vary from 8.07 to 7 for control, 7.92 to 6.6 for SC based fortified yoghurt (1%) and 7.91 to 6.5 for WPI based fortified yoghurt 1%. This might be because of resveratrol content released from encapsulated components which gave bitter taste. Further, SC based fortified yoghurt (1%) showed less syneresis as compared to WPI based fortified yoghurt (1%) which affected

overall acceptability of sensory scores. Mean scores for colour and appearance, body and texture, and flavour decreased significantly ($p \leq 0.05$) with increasing storage period. Spoilage of cultured dairy products is primarily caused by excessive fermentation, enzymatic breakdown or contamination with undesirable microorganisms. The low pH of yoghurt may offer a selective environment for growth of acid tolerant yeasts and moulds. Psychotropic organism may also pose a serious problem during storage at low temperature and result in defects like whey separation, ropiness, high acidity, gas formation and curdy body formation during storage. Over all, during storage the scores for overall acceptability were decreased significantly between storage period and samples.




Average value n=6.
Mean with different superscript differs significantly ($p \leq 0.05$) and all the values are expressed as (mean ± SE). Small letter shows difference between the samples while capital letter shows difference between the days.

**Fig 4:** Changes in Overall acceptability of functional yoghurt prepared by Incorporation of encapsulated resveratrol powder during storage

**Changes in microbiological parameters of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage *L. bulgaricus and S. thermophilus* counts**

During storage, the *L. bulgaricus* counts decreased significantly ($p \leq 0.05$). On 0 day the counts for SC based optimised yoghurt and WPI based optimised yoghurt was 6.92 log cfu/gm and 6.9 log cfu/gm respectively and on the 3rd day the counts was reduced to 6.84 log cfu/gm and 6.82 log cfu/gm with a reduction of 0.08 log cfu/gm and 0.08 log cfu/gm respectively. On 15th day the reduction rate was about 0.63 log cfu/gm and 0.47 log cfu/gm respectively.

During storage, the *S. thermophilus* counts are significantly ($p \leq 0.05$) reduced. On 0th day SC based optimised yoghurt and WPI based optimised yoghurt shows 7.05 log cfu/gm and 6.94 log cfu /gm respectively 3rd day shows 6.93 and 6.87 log cfu/gm, with a reduction of 0.12 log cfu/gm and 0.07 log cfu/gm. On 15th day the reduction was 0.53 and 0.5 log cfu/gm, respectively in SC based and WPI based functional yoghurt.

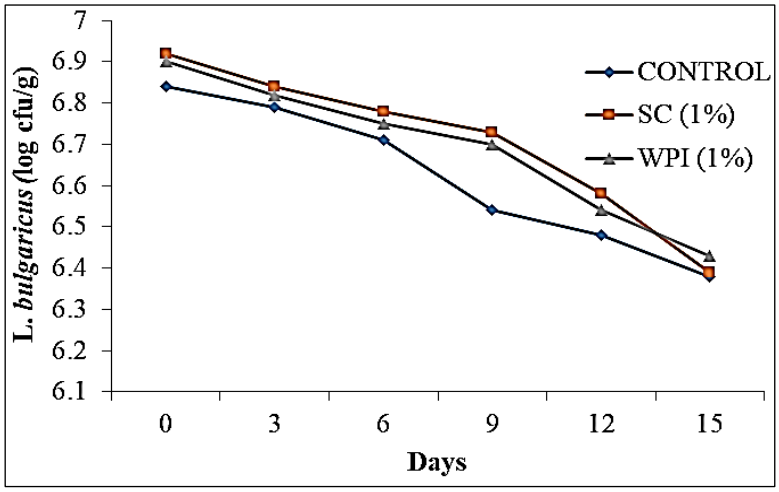



**Fig 5:** Changes in *L. bulgaricus counts* of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

Survival, as expected higher in refrigerated samples, and there was a relation between the decrease of pH and the decrease of bacterial counts. The high acidity is the main reason for the lower survival of lactic acid bacteria in fermented products with very low pH values. Also there was significant difference within the sample with respect to pH and acidity,

because of different composition of yoghurt. Therefore, the present findings are in correlation with the findings of (Joon *et al*., 2018) [12].

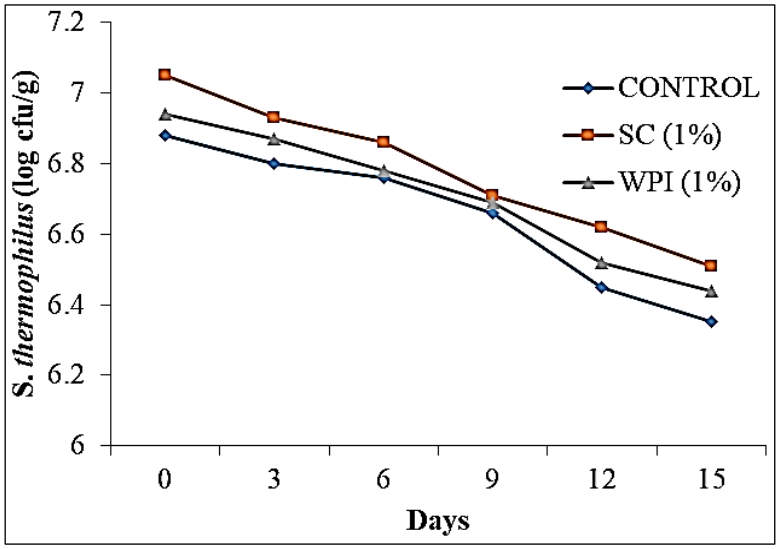



**Fig 6:** Changes in *S. thermophilus* count of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

**Coliforms**

During storage, the coliforms count was zero, indicating good hygiene practices followed during product manufacture.

**Yeast and Mould count**

During storage conditions, upto third day there was no growth of yeast and mould was observed. From 6th day onwards slight growth of yeast and mould was detected. The counts varied significantly ($p \leq 0.05$) during storage. On 3rd day 15 cfu/ gm was observed for SC based optimised yoghurt and 17 cfu /gm was observed for WPI based optimised yoghurt. On 15th day the growth was 95 cfu/gm for SC and 92 cfu/gm for SC. The counts increased significantly during storage and also showed significant difference among the sample. The growth was observed may be because of air born contamination. Upto 15th days the growth was less than the prescribed standards of FSSAI (2011) [13], therefore the product is safe to consume.

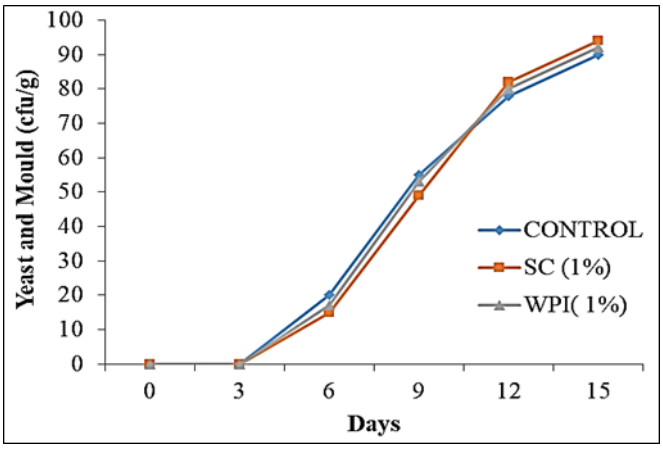



**Fig 7:** Changes in yeast and mould count of functional yoghurt prepared by incorporation of encapsulated resveratrol powder during storage

The microbiological analysis of the yoghurt samples during storage revealed that control yoghurt and optimised yoghurt was acceptable up to 15th days. After this period, coliform and yeast & mould counts were increased beyond the prescribed limit of FSSAI standards (2011). The microbial counts correlated with the sensory scores values.

**Conclusion**

From the studies, it was concluded that the storage period of control and experimental yoghurt samples showed significant effect on sensory characteristics like colour and appearance, body & texture, flavour and microbiological parameters. All the microbiological scores were within the limit and acceptable up to 15 days.

**Conflict of Interest:** Authors are not having any conflict of interest for publishing this article in this journal.

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