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# **Physico-chemical, microbiological and sensory characteristics of stirred yoghurt supplemented with myrtle berries**

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# INTRODUCTION





- Myrtle (*Myrtus communis* L.), an evergreen shrub belonging to the family Myrtaceae, is distributed in Europe, Asia, Africa and America. The leaves and berries are widely used in the Mediterranean area to flavour meat and fish dishes, as well as an aroma for wines and liqueurs (Anon, 2006). It is also used in traditional medicine and the perfumery, cosmetic and pharmaceutical industries.
- Myrtle (*Myrtus communis* L.) is endemic in the Mediterranean area and has long been used by locals for its culinary and medicinal properties (Atzei, 2003). Myrtle leaves and extracts have been studied, but only recently the berries have been the object of scientific interest.

- Myrtle berries are well known in some Mediterranean areas since they are used for food aromatisation, and to prepare a typical liqueur, which has been recognised by the European Community in 2007. The antioxidant properties and composition of the ethanol extracts obtained from myrtle berries have been studied (Alamanni & Cossu, 2004; Montoro et al., 2006a; Vacca, Piga, Del Caro, Fenu, & Agabbio, 2003), mainly focusing on compounds like anthocyanins and flavonols (Alamanni & Cossu, 2004; Franco et al., 2002; Montoro et al., 2006a, 2006b), which are thought to be responsible for their antioxidant effects.
- The fruits of this plant are mostly composed of volatile oils, tannins, sugars, flavonoids and organic acids such as citric and malic acids (Baytop, 1999; Martin et al., 1999).



- In this research the effect of the addition of myrtle berries( 5, 10,15% w/w) on the

- fermentation kinetics
- texture parametres(syneresis and WHC)
- antioxidant activity
- post acidification
- sensory profiles
- microbial counts

of stirred yoghurts were determined during 21 days of refrigerated storage.

**The purpose of the inclusion of myrtle berries in yogurt aims to increase its antioxidant activity and functionality.**

# MATERIAL AND METHODS



# Preparation of Yoghurts and Myrtle Marmalade



A 12%(w/v) solids non fat milk was standardised by using skim milk powder and divided into four equal portions. The milk was pasteurised at 90C for 10 min, then cooled to 43C and inoculated with yoghurt culture. Incubation took approximately 4.5 h at 43 C to reach a pH value of 4.6-4.7. After precooling at ambient temperature, the yoghurt samples were stored at 4C for 12 h. Meanwhile, for the marmalade, myrtle berries were blanched at 85 °C for 10 min, and subsequently mashed through a sieve of 1.5 mm mesh size. Finally, sugar was added at levels of %50.

Plain yoghurts fortified with myrtle marmalade at levels of 0% (control group), 5%, 10%, and 15% (w/ w) and then analysed throughout the storage period (1., 7.,14. and 21. days).

# Physico-Chemical Analysis

## pH values

- The pH of both the plain and each of the stirred fruit yogurts were measured weekly using a 315i /SET pH-meter and combined sentix 42 electrode (WTW, Weilheim, Germany).

## Water holding capacity

- The water-holding capacity was determined by a procedure adapted from Isanga & Zhang (2009).

## Colour measurement

- Colour measurement was performed using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). The  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$ (yellowness) colour measurements were determined.



# Physico-Chemical Analysis

- Antioxidant activity by DPPH inhibition assay

DPPH inhibition was determined as described by Shetty et al.(1995).

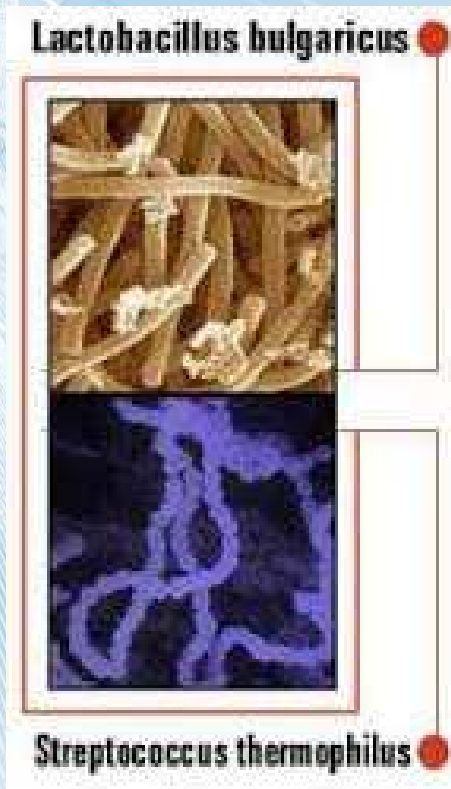
- Syneresis

It was determined by the method reported by Isanga and Zhang (2009).

- Total Solids Content

The total solid contents of the yoghurt samples were estimated according to Association of Official Analytical Chemists methods (AOAC 2002).

# Microbiological Analysis



- The samples of plain and stirred fruit yogurts in refrigerated storage were used to enumerate microorganisms from the day of manufacturing up to 3 weeks storage, at weekly intervals. Colonies from the incubated plates were counted using a colony counter.
- M-17 agar (Oxoid Australia Ltd) was used for the selective enumeration of *S. thermophilus* (Dave & Shah, 1996).
- For the selective enumeration of *L. delbrueckii subsp. bulgaricus* pH modified (4.58) MRS agar was used (Merck).

# Sensory and Statistical Analysis

- Sensory characteristics were determined by 10 experienced panelists. The score given by panelists for each attribute of a sample was noted separately. The qualities judged were: odour, taste, mouth feel, colour, texture, acidity and overall acceptability (0-5 points) (Anonymous 1989).
- Data were subjected to analyses of variance (ANOVA) and Student's t comparison test by using the Statistical software JMP 5.0.1 (SAS Institute).



# RESULTS AND DISCUSSION



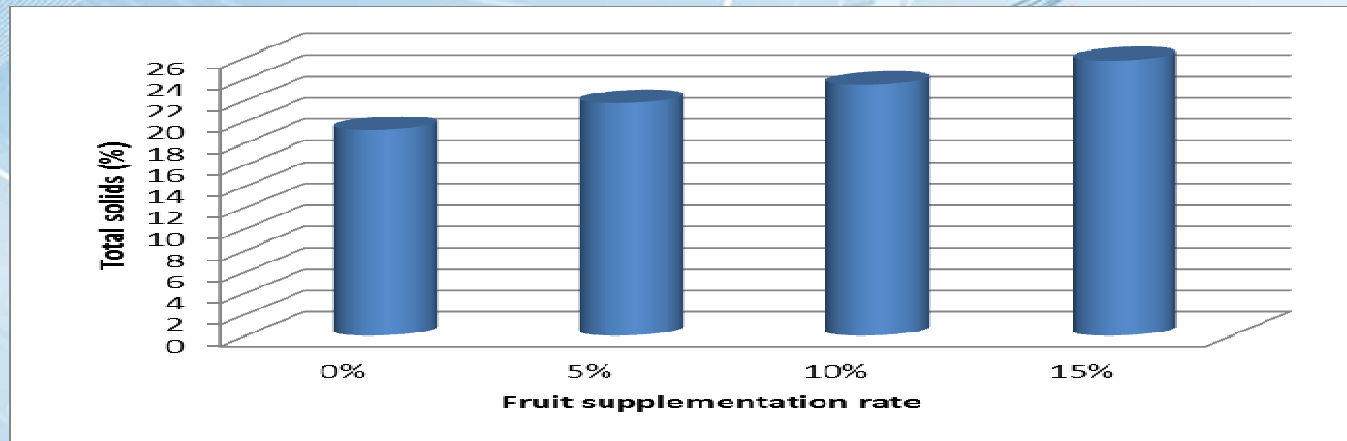
# Statistical Analysis

	0%	5%	10%	15%
pH	4,53±0,002 <sup>a</sup>	4,51±0,002 <sup>b</sup>	4,49±0,002 <sup>c</sup>	4,46±0,002 <sup>d</sup>
Syneresis	39,65±0,25 <sup>a</sup>	37,84±0,25 <sup>b</sup>	36,10±0,25 <sup>c</sup>	35,26±0,25 <sup>d</sup>
Water Holding Capacity	61,58±0,21 <sup>d</sup>	67,20±0,21 <sup>c</sup>	70,30±0,21 <sup>b</sup>	72,88±0,21 <sup>a</sup>
Antioxidant activity	28,58±0,09 <sup>d</sup>	33,79±0,09 <sup>c</sup>	36,90±0,09 <sup>b</sup>	38,95±0,09 <sup>a</sup>
Colour (L)	95,67±0,23 <sup>a</sup>	92,79±0,23 <sup>b</sup>	90,60±0,23 <sup>c</sup>	88,53±0,23 <sup>d</sup>
Colour(a)	-3,11±0,03 <sup>d</sup>	-1,63±0,03 <sup>c</sup>	-0,77±0,03 <sup>b</sup>	-0,08±0,03 <sup>a</sup>
Colour(b)	7,60±0,13 <sup>c</sup>	7,79±0,13 <sup>c</sup>	8,38±0,13 <sup>b</sup>	8,93±0,13 <sup>a</sup>
<i>Lb. delb.bulgaricus</i> counts(10 <sup>6</sup> cfu/ml)	1,75±0,04 <sup>d</sup>	2,29±0,04 <sup>c</sup>	3,02±0,04 <sup>b</sup>	4,24±0,04 <sup>a</sup>
<i>S. thermophilus</i> counts (10 <sup>8</sup> cfu /ml)	4,74±0,048 <sup>d</sup>	6,04±0,048 <sup>c</sup>	6,73±0,048 <sup>b</sup>	7,82±0,048 <sup>a</sup>

Mean (±SE)

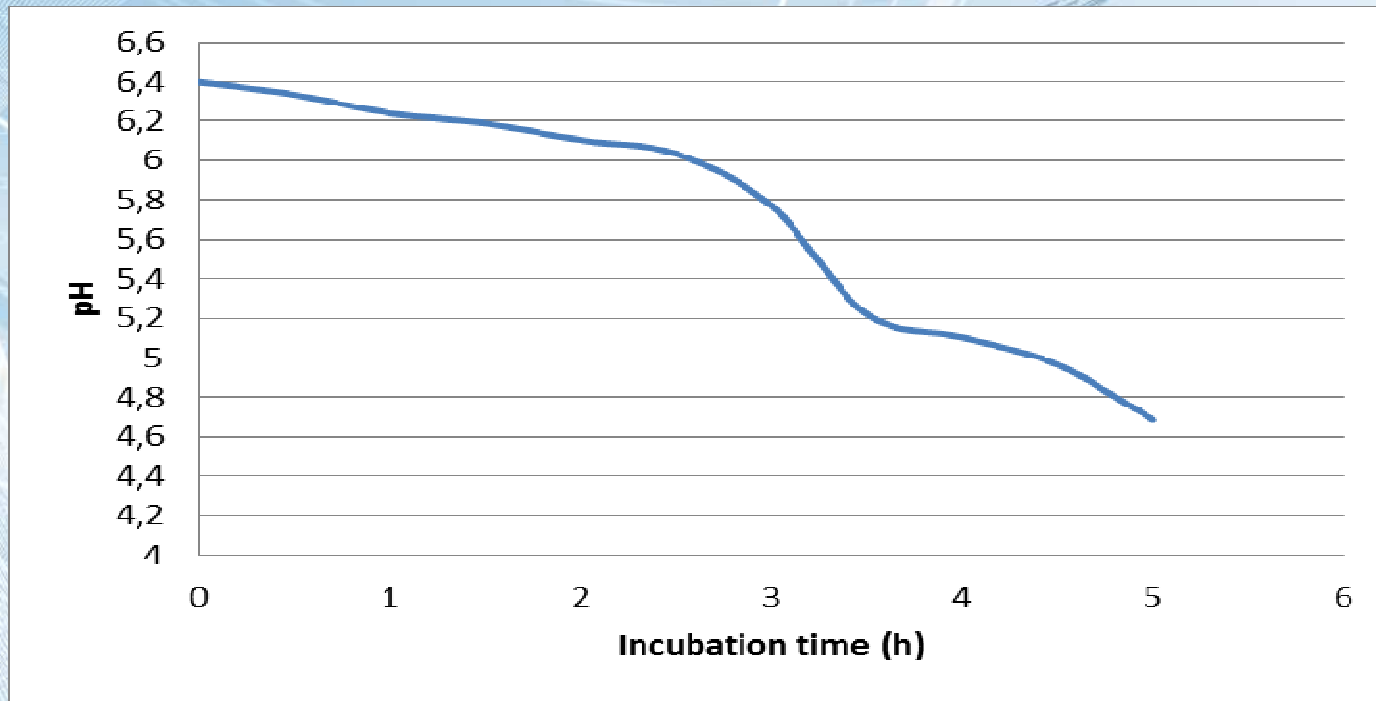
a,b,c,d Values in the same row having different superscripts differ significantly (p < 0.01)

## Total Solids Content



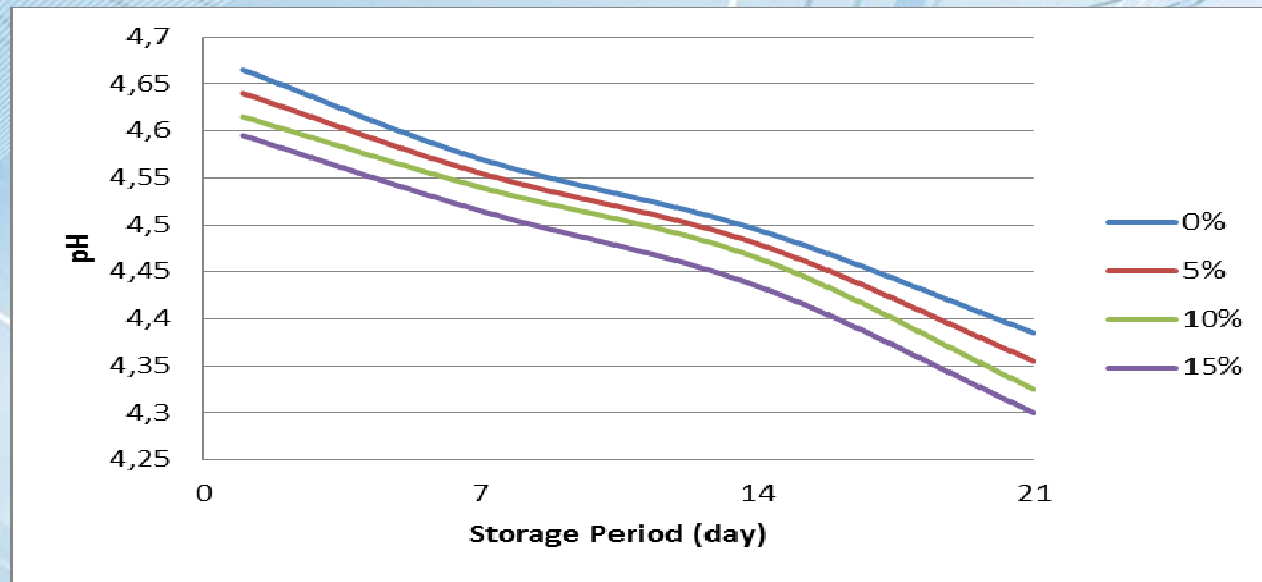
- The content of total solids of plain yoghurt was around 19,21 %, while with myrtle marmalade was 25,71%.
- The total solid contents of the yoghurt samples significantly increased with fruit addition ( $P < 0.01$ ).
- The highest total solid contents of yoghurt samples was determined in the group produced with 15% myrtle marmalade addition.

# pH Development



- The pH of yoghurt milk decreased from 6.4 to 4.6 during fermentation period. The pH of milk decreased to 4.6–4.7 in 5 h incubation.

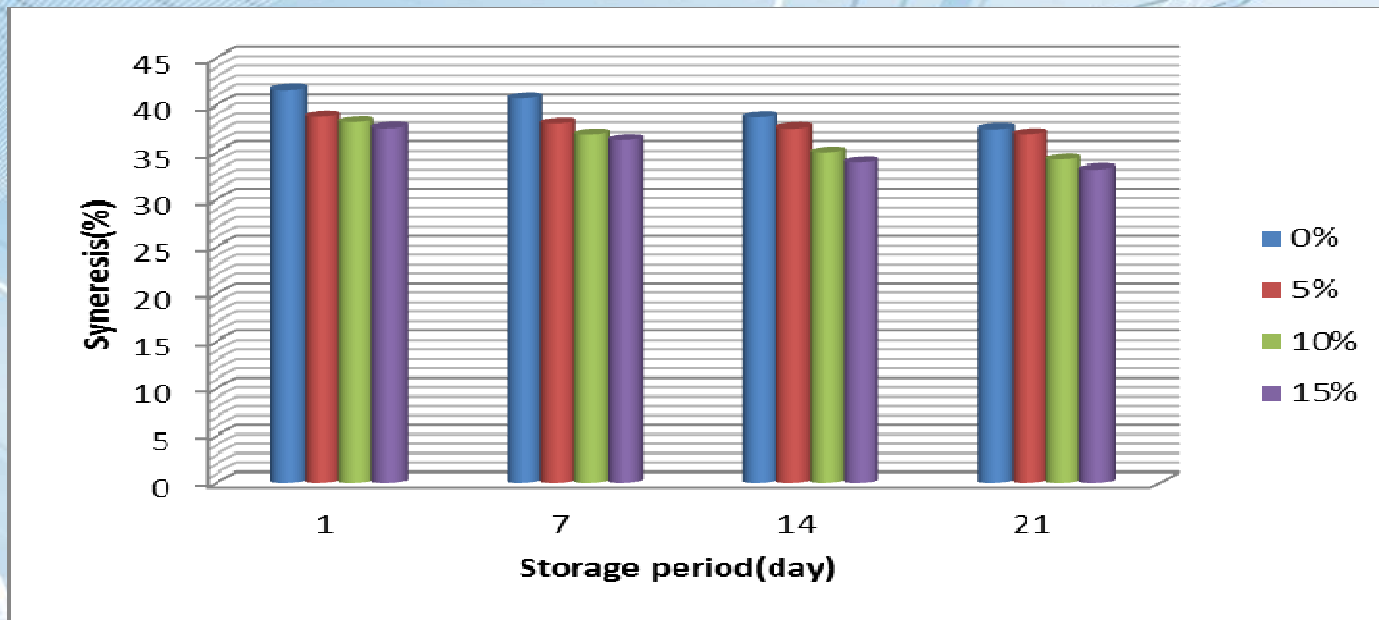
# Post Acidification



- The average pH of samples decreased from 4.6 to 4.3 after 21 days storage. Refrigerated storage is decreased pH values in both types of yoghurt. This pH decline may have been due to continued fermentation by the lactic acid bacteria and the contribution of the acidity of the added myrtle marmalade. Similarly, Vedamuthu(1982) reported that extended refrigeration to 28 days decreased the pH of yogurts to lower pH values (4.2-4.4) possibly as a result of accumulation of acetic acid, acetaldehyde, formic acid and lactic acid.
- The pH values of fruit added samples were lower than the control group and higher concentrations of myrtle marmalade led to lower pH values in yoghurt.

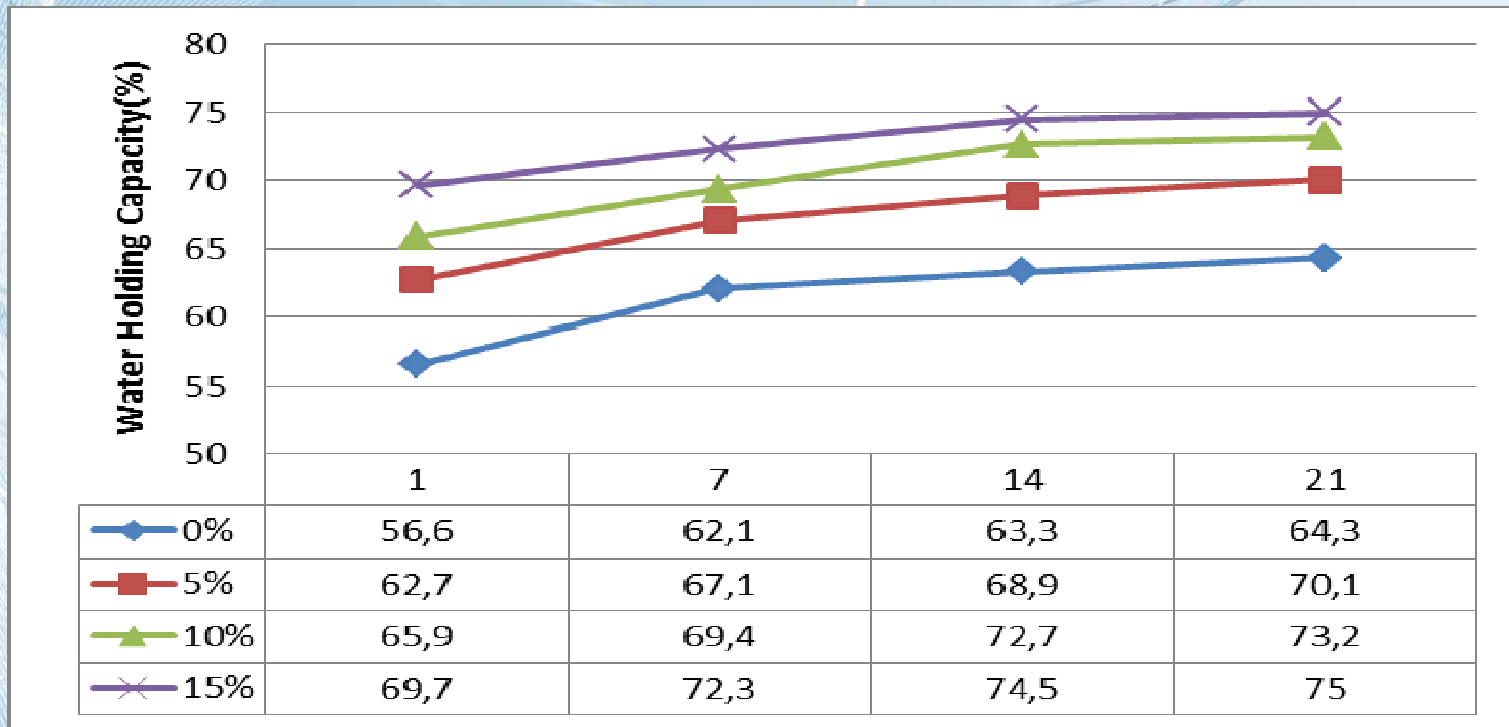


# Syneresis



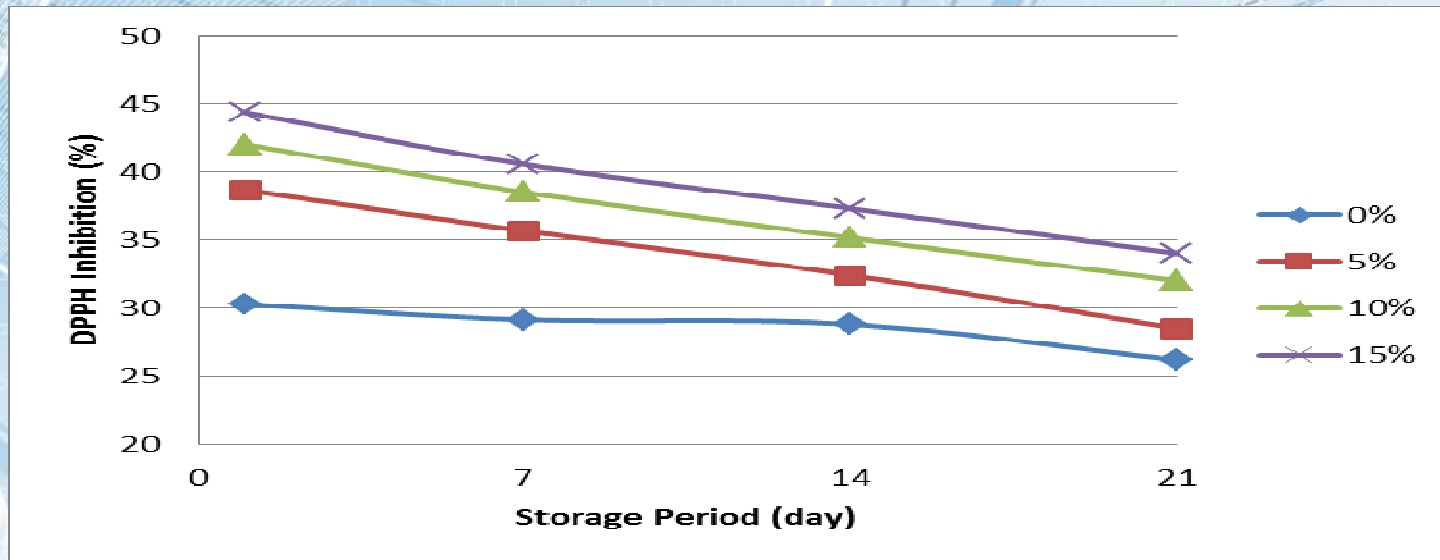
- Syneresis, an undesirable property in yogurt products, is the effect of liquid separating from the yogurt curds (Wu, Hulbert & Mount, 2001).
- Yogurts fortified with 15% myrtle marmalade resulted in the lowest syneresis during 21 days. Syneresis was found to be significantly higher in plain yogurt than in the stirred fruit yogurts regardless of the added marmalade levels, probably due to its higher total solids. So addition of myrtle fruits significantly decreased syneresis ( $p < 0,01$ ). These results are confirmed with other study such as Shori and Baba(2011a, b, c), Amirdivani and Baba(2011) and Zainoldin(2009).

# Water Holding Capacity



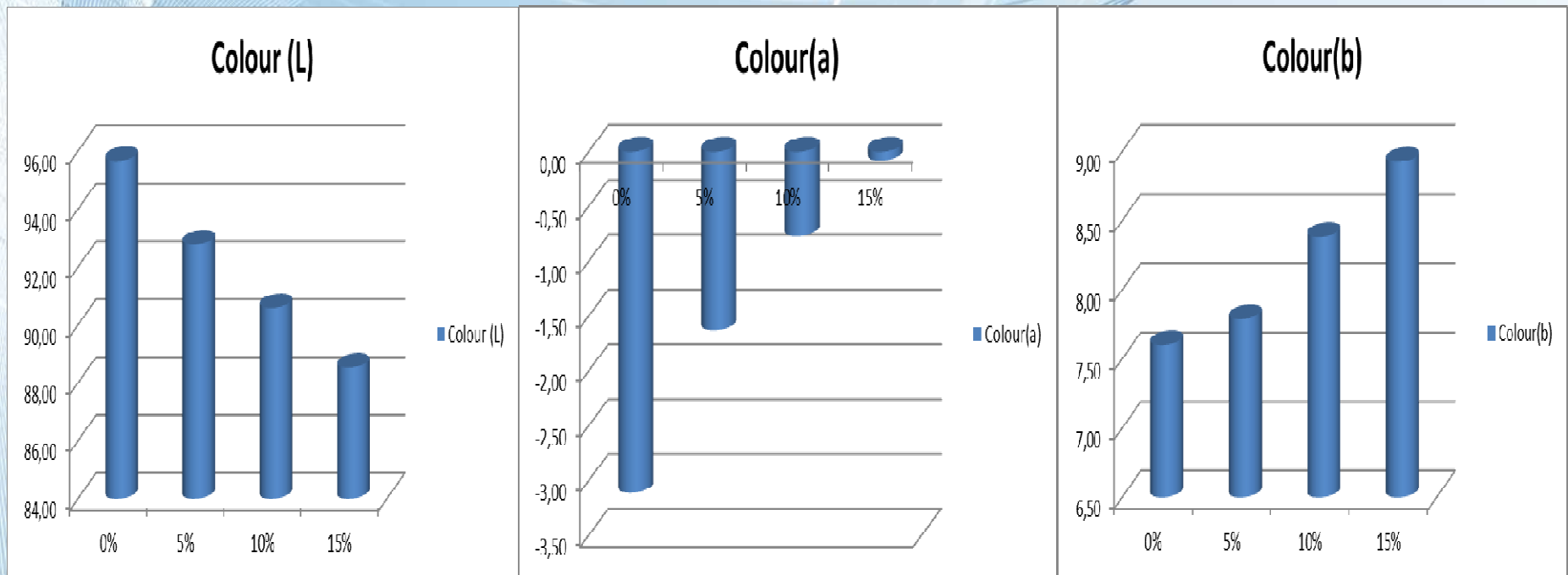
- In contrast to syneresis (as an important defect), WHC is also one of the most important physical properties, i.e., the higher the value, the better the curd stability(Srisuvor,2013).
- In this study, plain yogurts demonstrated significantly lower water holding capacity compared to stirred fruit yogurts.The WHC in 15% fruit supplemented yoghurts were significantly higher than all other samples( $p < 0,01$ ).

# Antioxidant Activity



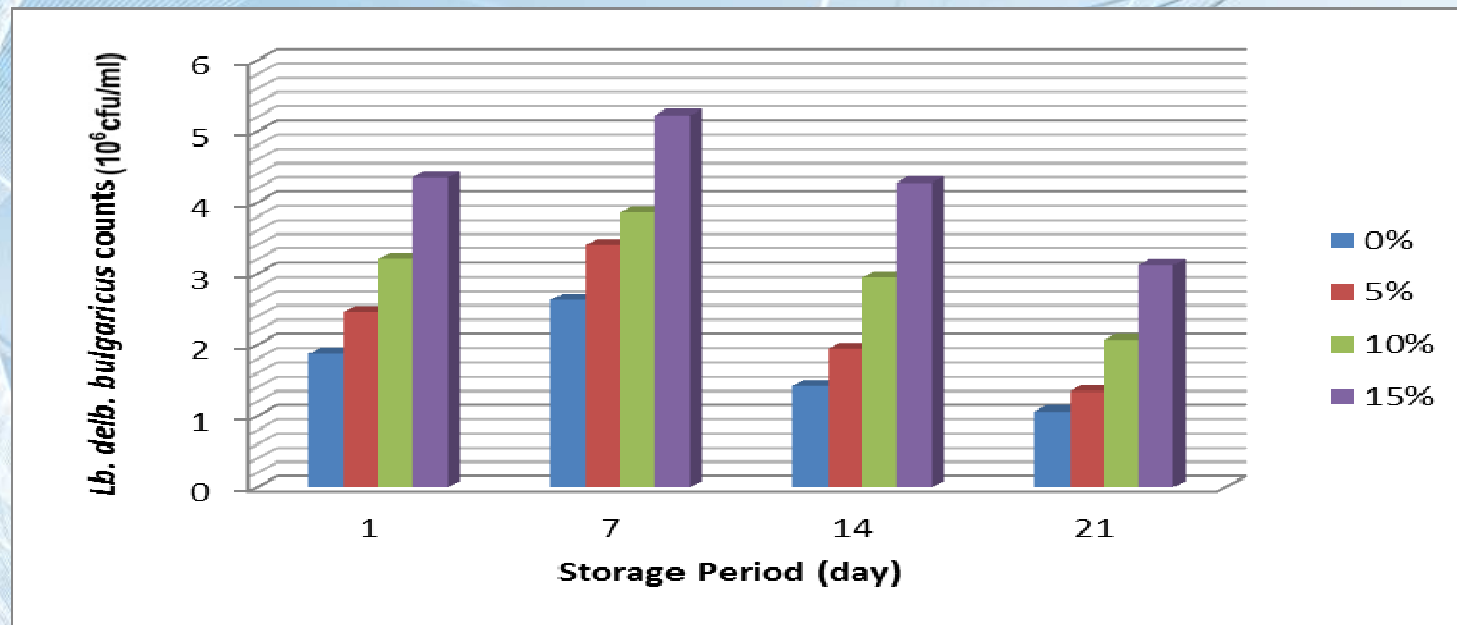
- Antioxidant capacity of yoghurts supplemented with fruits were higher than in their controls. Highest antioxidant activity was monitored in yogurts produced with 15% myrtle marmalade addition. The high liberation of phenolic compounds in the presence of myrtle marmalade in yoghurt may be contributed to higher antioxidant activity than in the absence.
- DPPH inhibition was slightly decreased during storage period. The reduction in antioxidant activities during refrigerated storage of yogurt is attributed to increasing degradation of phenolic compounds with antioxidant activities (Yildiz & Eyduran, 2009) and/or increasing milk protein-polyphenol interaction (Yuksel, Avci, & Erdem, 2010). Also, interaction between marmalade addition rate and storage period was found to be significant ( $p < 0.01$ ).

# Colour



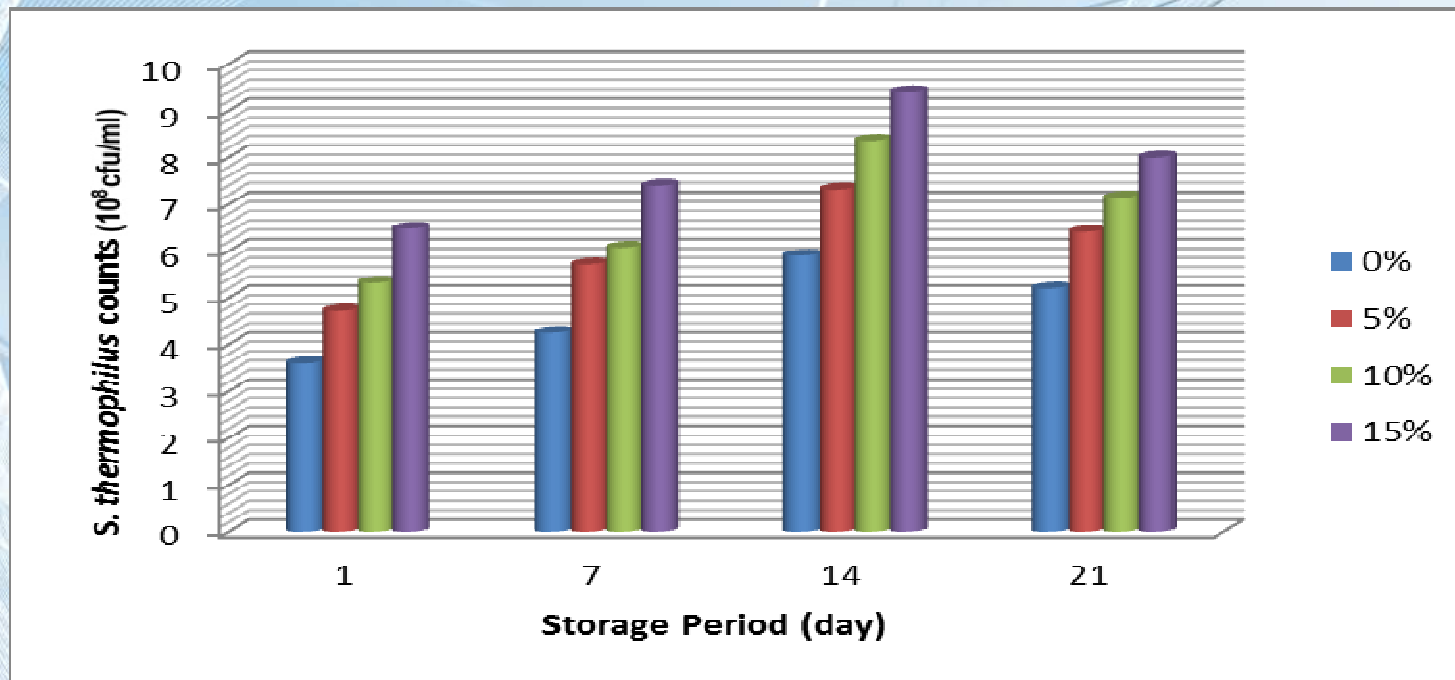
- With respect to color redness(a) and yellowness(b) values were increased depending on the ratio of myrtle marmalade added whereas lightness(L) values were decreased.
- There is an interaction between fruit addition rate and storage period in 'a' values(  $p < 0,01$ )

# *Lactobacillus delbrueckii subsp. bulgaricus* Counts



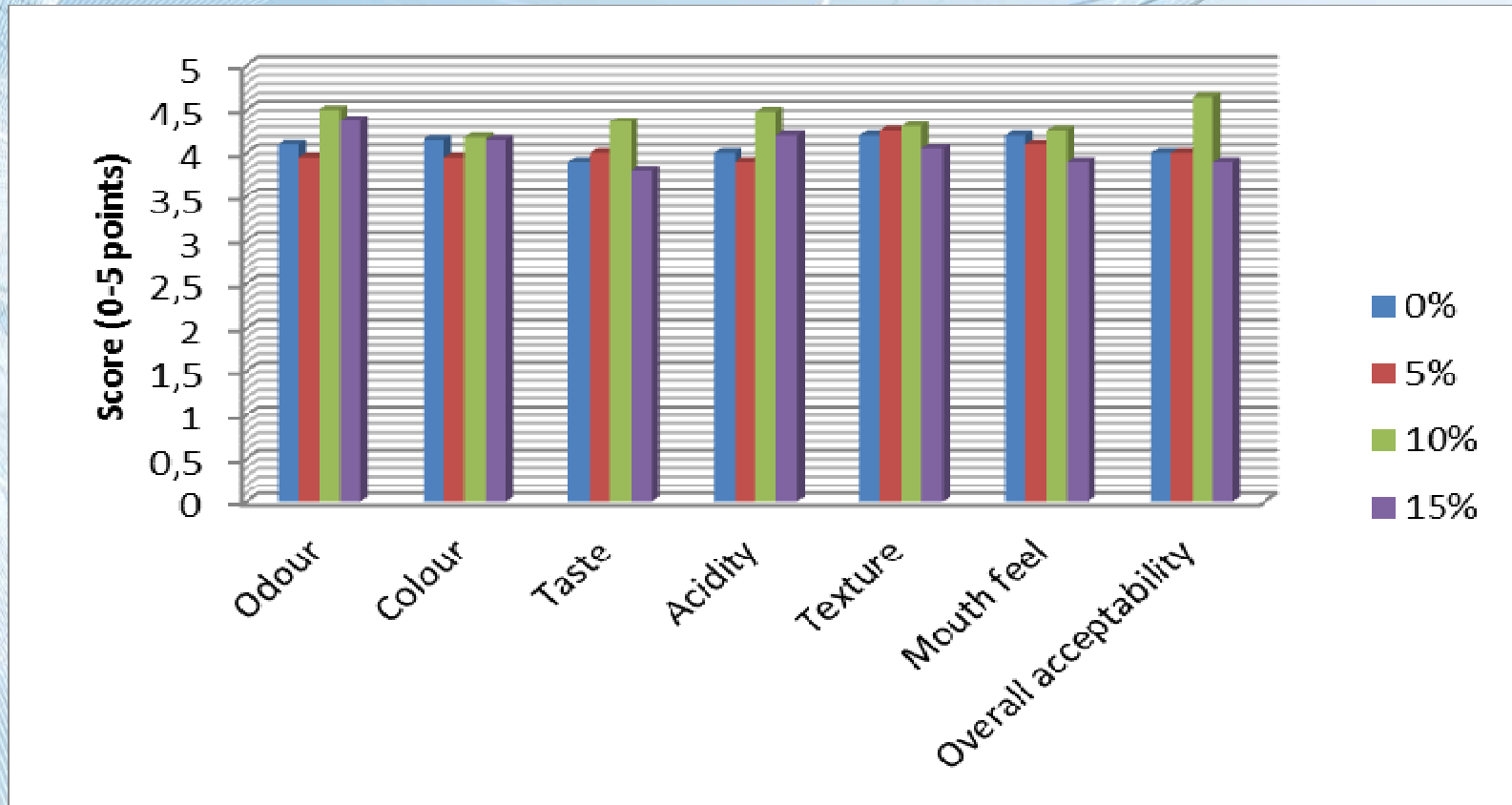
- The viable counts of *Lactobacillus spp* for both types of yogurts were increased throughout the first 7 days. The reduction of *Lactobacillus spp* counts between 7-21 days could be associated with the post acidification of yogurt which causes a further reduction in pH values (Shah, 2000; Omer and Eltinay, 2009; Eissa et al., 2010).
- Counts of *L. delbrueckii subsp. bulgaricus* were found to increase significantly depending on fruit addition rate ( $p < 0,01$ ). Also, interaction between fruit addition rate and storage period was found important ( $p < 0,05$ ).

## *Streptococcus thermophilus* Counts



- The increase in the viability of *S. thermophilus* in both plain and myrtle marmalade supplemented yogurts throughout the first 14 days of refrigerated storage was in agreement with other previous studies (Biorollo et al., 2000).
- *S. thermophilus* counts of the yoghurt samples significantly increased with fruit addition ( $P < 0,01$ ). Also, there is an interaction between fruit addition rate and storage period ( $p < 0,05$ ).

# Sensory Analysis



Among the tested various yoghurt samples, addition of 10% myrtle marmalade resulted in the highest scores for overall sensory attributes.

# CONCLUSION





- Addition of myrtle marmalade to yoghurt has affected the growing and liveliness of the characteristic starters in the storage period. *Lactobacillus delbrueckii subsp. bulgaricus* and *S. thermophilus* values increased with fruit addition compared with the control group samples.
- Control group showed the highest brightness, while 15% fruit containing samples had the lowest brightness values. The 'a' and 'b' values increased in the yoghurt samples produced with myrtle marmalade addition.
- **The results obtained from this study showed that myrtle fruits have a potential to play a role in increasing antioxidant activity, water holding capacity and reducing sineresis in yoghurts. In addition to these, addition of myrtle berries appeared to improve the sensory characteristics of yoghurts.**



*Thank you for  
your patience*