

# Organic acid and carbohydrate changes in carrot and wheat bran fortified set-type yoghurts at the end of refrigerated storage

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**Abstract:** The effects of using of wheat bran (1%), carrot (1%) and wheat bran (1%) +carrot ( 1%) as a supplement on pH, organic acids, carbohydrates and viscosity of yoghurt as well as overall acceptability were investigated. Fortification of milk with wheat bran resulted in a significant (  $P<0.01$ ) increase in oxalic, orotic, pyruvic, lactic, formic, acetic, propionic and hippuric acid contents, and a decrease in total sugar content of yoghurt. The lowest level of total organic acid and a slow post acidification rate were observed for yoghurt made with carrot which was much more preferred by panelists at the end of refrigerated storage. Fortification also led to an increase in viscosity of yoghurts. At the end of storage, there was a significant ( $P<0.05$ ) increase in viscosity of control yoghurt, whereas fortified yoghurt did not any change. Increases in lactic acid (from 9084 to 10823  $\text{mgkg}^{-1}$ ) and formic acid (from 665 to 802  $\text{mgkg}^{-1}$ ) contents of control yoghurt resulted in a significant ( $P<0.05$ ) decrease in overall acceptability score (from 7.5 to 6.2). It was concluded that yoghurt samples with wheat bran ( 1%) and wheat bran ( 1%) +carrot ( 1%) had the highest total organic acid content (about 14200  $\text{mgkg}^{-1}$ ) and titratable acidity (about 1.85% as lactic acid) were received the lowest overall acceptability score ( 5.6 out of 9) by panelists.

**Keywords:** Yoghurts, Organic Acids, Sugars, Wheat Bran, Carrot

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## 1. Introduction

Today, consumers have a wide variety of dairy foods to meet their taste, nutrition, health, and convenience needs. Culture-containing dairy foods such as yoghurt are an excellent source of many of milk nutrients and also they may offer health benefits [1] for hypercholesterolemia and certain gastrointestinal conditions [2]. These beneficial effects are due to their mainly organic acid contents since organic acids tend to exert preservative effect by controlling the growth of contaminating spoilage and pathogenic organisms [3]. Lactic acid fermentation also contributes to improved storage qualities, physical properties and flavour. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health [4].

Consumers have been recently concerned about fiber-rich foods due to their healthy reasons. By products like

wheat bran are “nondigestible” which is called as prebiotics. Wheat bran has dietary fiber (43.2%), cellulose (8.7%), hemicellulose (28.3%), lignin (3.2%), pectin (3.0%) moisture (10.3%) and nitrogen (2.64%). Wheat bran and its fractions have recently been reported to be strong anticarcinogens for colon carcinogenesis in rats [5]. Carrots are now increasingly consumed, mainly due to their pleasant flavour and perceived health benefits related to their vitamins, minerals and dietary fibre (about 31.99 % crude fiber) and well-known antioxidants such as terpenoid  $\beta$ -carotene [6]. The optimal dietary manipulation could be a good alternative in preventing, mainly carcinoma diseases. One of the ways of preventing diseases may be the together use of lactic cultures and natural prebiotics. Studies on the addition of fruits or byproducts having high fiber content to milk prior to fermentation process are scarce. Only a few studies have been carried out on conventional fiber fortified yoghurt and flavoured milk drink [7,8]. In these studies, sweetened plain yoghurt or milk were produced. Therefore, the objective of this study was to

determination the quality properties of yoghurts fortified with wheat bran (1%), carrot (1%) and carrot (1%) + wheat bran (1%). There will also be investigated the effects of using of wheat bran and carrot as a supplement on lactic acid fermentation. As quality attributes, we performed to acidity, organic acid, apparent viscosity and overall acceptability, which are important parameters in acceptability of products. So the use of by products of cereal industry in yoghurt product will be investigated.

## 2. Materials and Method

### 2.1. Materials

This study was carried out with cows' milk collected from Hatay province. YC-380 type commercial yoghurt culture containing *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* was used (Chr.Hansen-Peyma, Istanbul, Turkey). Wheat bran was used as nonpurified fiber source. Wheat was washed with top water and then was dried. After milling process to make wheat flour, wheat bran as a byproduct was obtained for yoghurt-making. Carrot (*Daucus carota* L.) roots with orange color were washed and brushed under running water. Washed roots were manually peeled (1mm), topped and tailed (1.0 -1.5 cm) using a sharp knife, and grated.

### 2.2. Yoghurt Production

Milk heated to 85°C for 30 min using batch pasteurization and cooled to 45°C in a water bath. Yoghurt culture inoculated at a ratio of 20g/100L<sup>-1</sup> milk for all the samples. Then milk was dispensed into Polystyrene plastic cups containing wheat bran (1%) (w/w), carrot (1%) w/w) and wheat bran (1%) (w/w) + carrot (1%) (w/w) , and incubated at 43°C. No fortified milk was control yoghurt. When yoghurt pH reached to 4.65, samples were removed from incubator, and transferred to a cold room at 4°C. Analyses were carried out at 1 and 21 days of storage at 4 °C. The samples were analyzed in quadruplicate at each sampling time, and the analysis was repeated if necessary. Three yoghurt trials were carried out on three successive weeks. Analyses were carried out using two samples from each type of yoghurt at each trial.

### 2.3. Chemical Analysis

Acidity was measured by titration with 0.1N NaOH, and expressed as a percentage of lactic acid [9]. The pH was determined with a pH meter (Thermo, Beverly, MA, USA).

Organic acids and sugars were analyzed according to the procedure described by Fernandez-Garcia and McGregor [10].

### 2.4. Viscosity

Apparent viscosity was measured with a Selecta rotational viscometer using Spindle No 4 at 10 rpm (J. P. Selecta, Barcelona, Spain). Viscosity measurements were

carried out at 4 °C, with the sample in a 250 mL beaker. Yoghurt was gently stirred for 20 s before analysis and triplicate measurements were conducted.

### 2.5. Sensory Analysis

Sensory evaluation was performed by 5 experienced panelists (two males and three female) who have been trained with yoghurt sensory scores characteristics. The panel consisted of academic staff and students from Food Engineering Department of Mustafa Kemal University, Hatay, Turkey. Yoghurts were removed from refrigerator (4°C) 1 h prior to sensory evaluation, kept at room temperature (22 ± 2°C). By using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely), consumers rated overall acceptability. Yoghurts were evaluated in duplicate by the panel members.

### 2.6. Statistical analysis

Statistical analysis of data obtained from yoghurts analysis for effects of two factors were performed using SPSS (Version 17.00) statistic program [11]. The factors were storage times (1 and 21 days) , types of yoghurt. Effects of storage and type of yoghurt were separately examined by one-way ANOVA, and the interaction between these factors was two-way analysis of variance (ANOVA). The mean differences were analyzed using Duncan's multiple-range test. The least significant difference ( $P < 0.05$ ) of the data is reported.

## 3. Results and Discussion

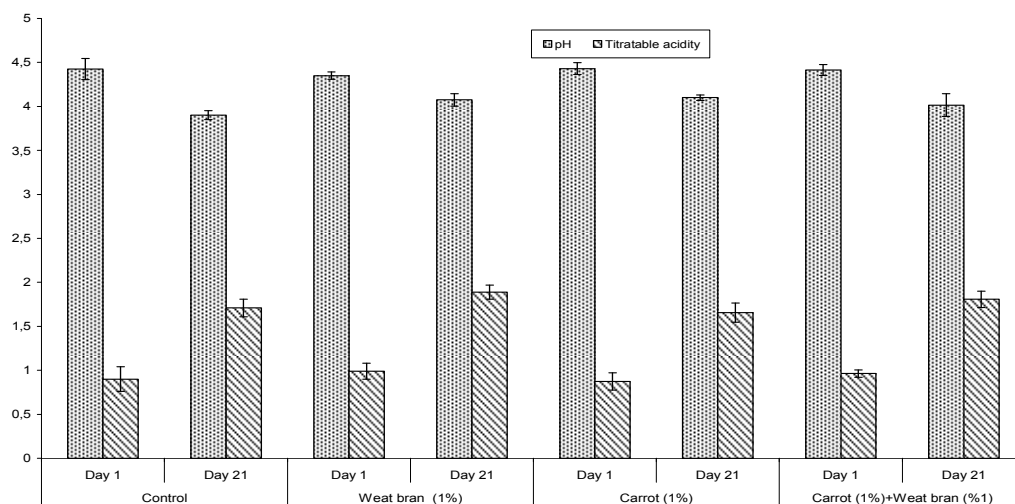
### 3.1. Chemical Composition for Raw Milk and Variation in Acidity

Cows' milk used for yoghurt manufacturing had 6.67 pH, 0.162 % titratable acidity, 12.04 % total solid, 3.40 % crude fat, 4.55% lactose and 3.60% crude protein. As noted by Tamime and Robinson [12], milk had the minimum milk solids-non-fat content [8.2 to 8.6 %] requiring for yoghurt-making.

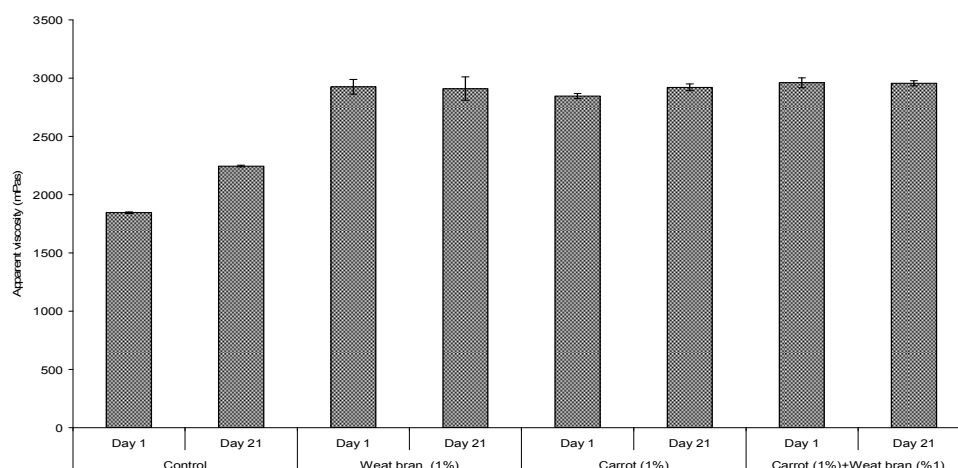
The incubation of yoghurt samples was ended at pH 4.65. The incubation time for control sample, milk fortified with wheat bran (1%) (w/w), carrot (1%) (w/w) and wheat bran (1%) (w/w) + carrot (1%) (w/w) was 319±11, 311±9, 337±12 and 333±5 min, respectively. Milk with wheat bran (1%) showed the shortest incubation time. Wheat bran could be led to a stimulatory effect on the growth of *Lactobacillus delbrueckii* supsp. *bulgaricus* which is mainly responsible for development of acidity [12]. At the beginning of storage, there were significant ( $P < 0.05$ ) differences in titratable acidity and pH values of yoghurt samples. As shown in Fig. 1., yoghurts made with wheat bran had the highest titratable acidity and the lowest pH with values of 0.99% (as lactic acid) and 4.35, respectively. This result can explain why have the shortest incubation time of yoghurt made with wheat bran. As expected, the

values of titratable acidity of the yoghurts significantly ( $P < 0.05$ ) increased at the end of storage. However, there were no significant changes in pH values of yoghurts except for control sample which showed a significant ( $P < 0.05$ ) decrease in pH at the end of storage (Fig. 1). Fortification

could be resulted in a buffering capacity in yoghurts since the significant increases in acidity of yoghurts fortified with wheat bran and carrot were not accompanied by strong decreases in pH.



**Fig. 1.** Changes in the titratable acidity (as lactic acid) and pH values of control sample and yoghurts fortified with wheat bran (1%) (w/w), carrot (1%) (w/w) and wheat bran (1%) (w/w) + carrot (1%) (w/w). The error bars are indicated standard deviation ( $n = 3$ ,  $P < 0.05$ ;  $P < 0.001$ ).



**Fig. 2.** Changes in viscosity of control sample and yoghurts fortified with wheat bran (1%) (w/w), carrot (1%) (w/w) and wheat bran (1%) (w/w) + carrot (1%) (w/w). The error bars are indicated standard deviation ( $n = 3$ ,  $P < 0.05$ ;  $P < 0.001$ ).

### 3.2. Evaluation of Organic Acids

The organic acids in raw milk and yoghurt samples are given in Table 1. During manufacturing and storage of yoghurts there was an appreciable change in the individual organic acid. As noted by Adhikari et al. [13], propionic, butanoic and hippuric acids were not identified in milk, but they found in all the yoghurt samples. Oxalic acid was found in neither milk nor control yoghurt. Citric acid, a product of body metabolism, was predominant organic acid in milk with a value of  $1773 \text{ mg kg}^{-1}$ . Citric acid accounted for about 86% of total organic acids in milk. The levels of organic acids identified in milk were similar to the values reported by Walstra and Jenness [14]. Concerning

yoghurts, lactic acid was the principle organic acid in all the samples and its amount ranged from  $90841 \text{ mg kg}^{-1}$  to  $10823 \text{ mg kg}^{-1}$ . The similar results were reported by Torre et al. [15]. At day 1, yoghurt made with wheat bran when compared with the other yoghurts contained lactic acid at the highest level ( $9826 \text{ mg kg}^{-1}$ ). This result was in consistent with the high titratable acidity, low pH value and the low lactose content of yoghurt made with wheat bran. As mentioned previously, wheat bran could have supplied nutrients and growth stimulatory factors for yoghurt culture. There was a significant ( $P < 0.05$ ) increase in lactic acid contents of other samples except yoghurt made with carrot at the end of storage.

**Table 1.** Organic acid and carbohydrate contents in milk, and carrot and wheat bran fortified yoghurts ( $n=3$ ).

	Control <sup>a</sup>			Wheat bran (1%) <sup>b</sup>		Carrot (1%) <sup>c</sup>		Carrot (1%)+Wheat bran (1%) <sup>d</sup>	
	Milk	Storage Days		Storage Days		Storage Days		Storage Days	
Acids (mg kg <sup>-1</sup> ) <sup>1</sup>		Day 1	Days 21	Day 1	Days 21	Day 1	Days 21	Day 1	Days 21
Oxalic	nd	nd	nd	9 <sup>b</sup>	10 <sup>b</sup>	2 <sup>a</sup>	2 <sup>a</sup>	8 <sup>b</sup>	10 <sup>b</sup>
Orotic	118	116 <sup>a</sup>	106 <sup>a</sup>	374 <sup>c</sup>	353 <sup>c</sup>	147 <sup>b</sup>	133 <sup>ab</sup>	311 <sup>c</sup>	331 <sup>c</sup>
Citric	1773	1726 <sup>ab</sup>	1689 <sup>a</sup>	1620 <sup>a</sup>	1667 <sup>a</sup>	1427 <sup>a</sup>	1584 <sup>a</sup>	1461 <sup>a</sup>	1596 <sup>a</sup>
Pyruvic	25	49 <sup>a</sup>	47 <sup>a</sup>	68 <sup>b</sup>	66 <sup>b</sup>	48 <sup>a</sup>	44 <sup>a</sup>	63 <sup>b</sup>	55 <sup>ab</sup>
Uric	20	22	22	25	26	23	22	23	23
Lactic	715	9084 <sup>a</sup>	10823 <sup>b</sup>	9826 <sup>b</sup>	10562 <sup>c</sup>	9289 <sup>a</sup>	9673 <sup>a</sup>	9374 <sup>ab</sup>	10611
Formic	782	665 <sup>a</sup>	802 <sup>ab</sup>	874 <sup>b</sup>	853 <sup>ab</sup>	760 <sup>a</sup>	721 <sup>a</sup>	824 <sup>ab</sup>	928 <sup>ab</sup>
Acetic	96	124 <sup>a</sup>	128 <sup>a</sup>	213 <sup>b</sup>	200 <sup>b</sup>	138 <sup>a</sup>	161 <sup>a</sup>	210 <sup>b</sup>	190 <sup>b</sup>
Propionic	nd	90 <sup>a</sup>	100 <sup>a</sup>	165 <sup>b</sup>	202 <sup>b</sup>	101 <sup>a</sup>	110 <sup>a</sup>	155 <sup>b</sup>	194 <sup>b</sup>
Butanoic	nd	201 <sup>a</sup>	218 <sup>a</sup>	230 <sup>b</sup>	239 <sup>b</sup>	237 <sup>a</sup>	189 <sup>a</sup>	221 <sup>a</sup>	243 <sup>b</sup>
Hippuric	nd	17	15	30	29	19	20	24	20
Total organic acid	3529	12094	13950	13434	14207	12191	12759	12674	14201
Sugars (mg kg <sup>-1</sup> ) <sup>1</sup>									
Lactose	42358	30121 <sup>c</sup>	28553 <sup>b</sup>	26239 <sup>a</sup>	25446 <sup>a</sup>	29614 <sup>c</sup>	28412 <sup>b</sup>	25947 <sup>a</sup>	25494 <sup>a</sup>
Glucose	169	2231 <sup>a</sup>	3152 <sup>b</sup>	2823 <sup>b</sup>	3585 <sup>c</sup>	2320 <sup>a</sup>	2378 <sup>a</sup>	2788 <sup>b</sup>	3642 <sup>c</sup>
Galactose	42	6906 <sup>a</sup>	7509 <sup>ab</sup>	7160 <sup>a</sup>	7641 <sup>ab</sup>	6847 <sup>a</sup>	7121 <sup>a</sup>	6611 <sup>a</sup>	7468 <sup>ab</sup>
Total sugar	42569	39258	39214	36222	36672	38781	37911	35346	36604

<sup>a</sup> Control yoghurt; <sup>b</sup> Wheat bran (1%) (w/w) fortified yoghurt; <sup>c</sup> carrot (1%) (w/w) fortified yoghurt; <sup>d</sup> carrot (1%) (w/w), and wheat bran (1%) (w/w) fortified yoghurt. SD; standart deviation. nd: not detected.

<sup>1</sup> Means for the the same parameters and treatment followed by different superscript letters are significantly different ( $P<0.05$ ).

Citric acid was the second most abundant organic acid in yoghurt samples. The fermentation of milk led to a significant [ $P<0.01$ ] decrease in citric acid contents of yoghurts fortified with wheat bran and carrot in comparison with control sample in which the negligible utilization of citric acid was observed during fermentation process and storage. This result is in accordance with the finding of Fernández-García and McGregor [8]. The amount of citric acid was slightly increased in yoghurts made with wheat bran and carrot at the end of storage. This could be attributed to the hydrolysis of wheat bran and carrot by yoghurt culture depending on the development of acidity. Formic acid was the third most abundant acid in all the yoghurts. As shown in Table 1, formic acid in milk was significantly ( $P<0.001$ ) utilized by yoghurt culture during fermentation process for control sample and yoghurt made with carrot. However, it showed an increase tendency in yoghurts with wheat bran in comparison with raw milk. It could be argued that yoghurt culture was probably used wheat bran as substrat for formic acid production. At the end of storage, no signifi-

cant changes in formic acid were observed for experimental yoghurts, but it increased significantly ( $P<0.05$ ) in control yoghurt. This result was in consistent with a significant reduce in pH of control yoghurt at the end of storage. Formic acid contents of yoghurts were similar to findings of Bevilacqua and Califano [16]. Orotic, uric and hippuric acids are nonprotein nitrogenous compounds in milk, all of which were found in the yoghurts, whereas hippuric was not detected in milk. Orotic and hippuric acids were significantly ( $P<0.001$ ) higher in yoghurts made with wheat bran than the other samples. This result was confirmed to the high level of titratable acidity of yoghurt with wheat bran since orotic acid was described as a growth factor for *L. delbrueckii* subsp. *bulgaricus* and also as a precursor for the synthesis of nucleotides [10]. Similarly, the concentrations of oxalic, propionic and butanoic acids were significantly ( $P<0.01$ ) greater in yoghurts fortified with wheat bran and wheat bran+carrot than control and samples with carrot. This situation may be related to wheat bran used which is probably contained the high levels of oxalic, orot-

ic and hippuric acids even though they were not detected since oxalic acid may produce from glycolate by glycolate oxidase in plants [17]. Among organic acids derived from mainly glucose, pyruvic acid showed the lowest level, ranged from 44 mg kg<sup>-1</sup> to 68 mg kg<sup>-1</sup> yoghurt. Pyruvic acid acts as substitute in various metabolic pathways for the mainly acetic and formic acids [18].

Acetic acid was significantly ( $P < 0.001$ ) increased in all the yoghurts in comparison with raw milk, but it did not change at the end of refrigerated storage. The concentration of acetic acid was significantly ( $P < 0.01$ ) higher in yoghurts fortified with wheat bran and wheat bran + carrot than control and carrot fortified yoghurts. In a previous study, the synthesis of acetic, butanoic and propionic acids was increased in the mediums containing wheat bran [19].

Overall, yoghurt with carrot had the lowest post acidification rate and titratable acidity was contained total organic acid at the lowest level (12759 mg kg<sup>-1</sup>) at the end of storage. This could be attributed to the inhibitory effect of fruit sugars on acidification rate of yoghurt culture [12].

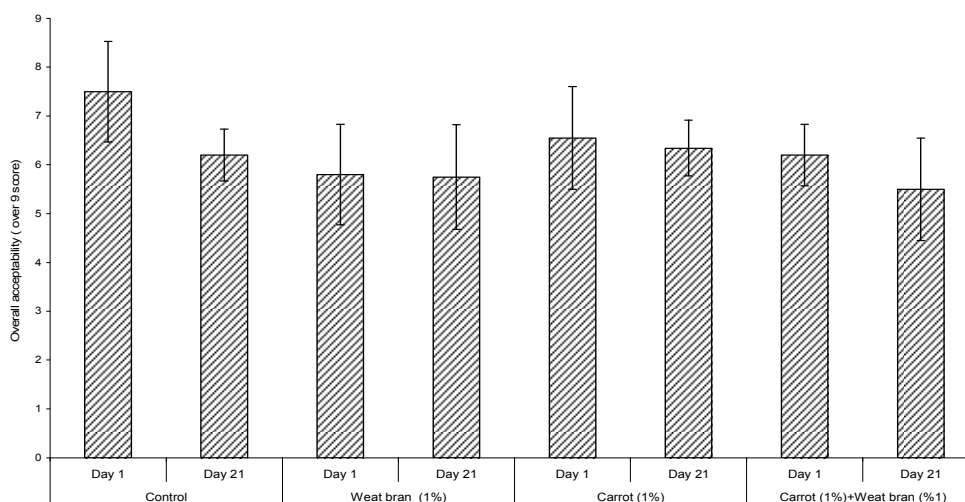
Of organic acids identified in yoghurts, in spite of the adverse effects of orotic and uric acids on gut flora, lactic, butanoic and acetic acids have a bacteriocidal or bacteriostatic effect. They suppress the multiplication of pathogenic and putrefying bacteria [20]. On the other hand, the most common kind of kidney stone is made of calcium oxalate which can irritate the gut and kidneys. Due to high oxalic acid content, long-term consumption of yoghurt fortified with wheat bran may lead to nutrient deficiencies

Concerning carbohydrates, the about 38 % of lactose in

milk was utilized by yoghurt bacteria in samples with wheat bran and wheat bran + carrot in comparison with the other yoghurt samples (about 30%). The sugars such glucose and galactose were significantly ( $P < 0.05$ ) increased in the yoghurt samples except yoghurt with carrot at the end of storage. The most decrease in lactose was observed for control yoghurt at the end of storage. This can explain that the break down of supplement is mainly responsible for the increase in acidity of yoghurts fortified with wheat bran since the highest level of total organic acids was observed for yoghurts with wheat bran.

### 3.3. Viscosity

As shown in Fig. 3., fortification of milk with wheat bran and carrot resulted in a significant ( $P < 0.05$ ) increase in apparent viscosity of yoghurts at the beginning of storage. This could be attributed to interactions between the hydrocolloids in wheat bran and carrot, and milk proteins. Typically an increase in viscosity would suggest a decrease in syneresis probably because of more water being held within yoghurts with carrot and wheat bran. At the end of storage, a significant ( $P < 0.05$ ) increase in apparent viscosity was observed for control yoghurt only. This could be attributed to the protein rearrangements in acid casein gels at refrigerated storage depending on the decrease in pH since a significant decrease in pH value and the most increase in titratable was observed for control yoghurt only at the end of storage.



**Fig. 3:** Overall acceptability scores of control sample and yoghurts fortified with wheat bran (1%) (w/w), carrot (1%) (w/w) and wheat bran (1%) (w/w) + carrot (1%) (w/w). The error bars are indicated standard deviation ( $n = 3$ ,  $P < 0.05$ ;  $P < 0.001$ )

### 3.4. Overall acceptability

At the beginning of storage, overall acceptability score (7.50 out of 9.0) was significantly ( $P < 0.05$ ) high in control yoghurt followed by yoghurts made with carrot, wheat bran+carrot and wheat bran. At the end of storage, the decrease in overall acceptability score of control yoghurt

was significant ( $P < 0.05$ ), whereas the other yoghurts did not any change. It is accepted that formic acid as well as lactic acid have a very important effect on the overall acceptability of yoghurt since these acids increased significantly ( $P < 0.01$ ) in control sample at the end of storage. In addition, overall acceptability is closely related to the amount of total organic acid and titratable acidity level

since yoghurts with high total organic acid (14200 mgkg<sup>-1</sup>) and titratable acidity (1.85%, as lactic acid) received the lowest overall acceptability score.

## 4. Conclusion

The lactic acid accounted for about 90% of the total organic acid content of yoghurts, followed in decreasing order by citric, formic, butanoic acid, orotic, acetic, propionic, hippuric, uric and oxalic acids. Fortification with wheat bran resulted in an increase in total organic acid, acidification rate and apparent viscosity, and a decrease in total sugar content as well as overall acceptability score of yoghurt. At the end of storage the least increases in total organic acid and titratable acidity were observed for yoghurt fortified with carrot. Therefore, yoghurt fortified with carrot may be suitable for long-time storage. Control yoghurt which had the lowest levels of lactic (9100 mg kg<sup>-1</sup>) and formic (665 mg kg<sup>-1</sup>) acids was much more preferred by panelists. In general, yoghurts had total organic acid content at above about 10000 mg kg<sup>-1</sup> showed a significant ( $P < 0.01$ ) decrease in overall acceptability score. It is worth noting that the use of carrot as a supplement when compared to wheat bran may be more suitable for yoghurt production since it led to a slow acidification rate, the less organic acid production and the higher overall acceptability in yoghurt at the end of storage.

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