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STUDY ON THE USE OF YOGHURT, WHEY, LACTIC ACID AND STARTER CULTURE ON CARROT FERMENTATION

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Keywords: fermentation, carrot, whey, lactic acid, starter culture

The effects of yoghurt, whey, lactic acid and starter culture on the fermentation of pickled carrots were examined. The fermentation process was observed by acidity and salt intake, whereas important factors for quality prediction as texture, odour, colour and taste were analysed *via* trained panellists. The spontaneous fermented samples were not preferred. Acid, starter culture, yoghurt and whey addition were effective on controlling fermentation as these favour the growth of lactic acid producing bacteria.

INTRODUCTION

A pickle is defined as an edible product that has been preserved and flavoured in a solution of brine and edible acid such as vinegar. The history of fermented vegetables extends so far into antiquity that no precise time can be established for its origin. The methods of preparation and preservation of vegetable substances presumably originated in the Orient [Altug, 1993; Babuchowski *et al*., 1999].

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and their antimicrobial products. Lactic acid producing bacteria have a major potential use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods. Nearly all vegetable material, including fruits handled like vegetables, such as cucumbers, tomatoes and olives, may be biopreserved by lactic-acid producing bacteria. They contain sugars and are nutritionally adequate as a substrate for the growth of the lactic acid bacteria and other microorganisms. The lactic acid producing bacteria are the acid producers; they are anaerobic, and they require a complex medium for growth. This is usually supplied from the nutrients withdrawn from the vegetable by the plasmatic action of salt. The lactic acid bacteria require small amounts of salt in the medium for optimal growth, as excess salt inhibits the growth [Ballesteros *et al*., 1999; Cemeroglu, 1992].

Fermentations involving vegetables are initiated by strains of *Leuconostoc mesenteroides* and continued by three other species of lactic acid bacteria. Lactic acid, carbon dioxide, ethyl alcohol, and acetic acid are major end products [Daeschel *et al*., 1987].

One must conclude that the characteristic flavour, aroma, and texture of fermented vegetables are dependent not only upon the nature of food itself, but also upon the changes resulting from the activity of microbial enzymes, enzymes indigenous to the food, and the interactions that occur during fermentation and subsequent curing or ageing [Desai & Sheith, 1997].

Pickles are preserved in part by acid, salt and sugar. The skilful blending of spices, sugar, salt, and vinegar with vegetable gives crisp, firm texture and pungent sweet, sour flavour. Pickles are generally grouped into three classes; dill pickles, sour pickles, and sweet pickles. However, the names applied vary from one manufacturer to the other, as each have their own formulas that have been developed over a period of years according to consumers' demands, climate and available raw materials [Daeschel *et al*., 1987; Desai & Sheith, 1997].

While a very important reason for pickling is to preserve the basic organic components from spoilage, of equal or greater importance are the resulting changes in sensory, physical and nutritional characteristics of the relatively bland starting materials. In pickled products spoilage and abnormal flavours have resulted in considerable losses. With acid and starter addition, growth of other fermenting microorganisms present naturally on the raw material can be eliminated or reduced under controlled fermentation process [Fleming, 1982, 1991; Fleming *et al*., 1985,1987].

Vegetables are pickled to preserve and prolong the shelf life by acid production with growth of lactic acid producing bacteria, either present in the microflora or added as starter culture. Therefore, the objective of the present experiment was to compare the effects of yoghurt, whey, lactic acid and starter culture on the acceleration of fermentation, shorten-

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ing fermentation time and enhancing the desired aroma, taste and colour of pickled carrots.

MATERIALS AND METHODS

Fresh carrots, purchased from a supermarket in Bursa, 1 day after the harvest, were washed to remove contaminants, such as dust, soil, and microorganisms. After washing, the carrots were cut into slices lengthways and/or widthways. The lengthway slices had a diameter of approximate 10 mm, and a height of 120–125 mm. The widthway slices had a diameter of approximate 30 mm, and a width of 20–25 mm. The slices were placed into 1 L hermetic jars (1:1, w slices/v brine), with 1% onion, 0.1% black pepper and 0.5% dill (Figure 1). The brining solution contained 5% salt, 0.3% sugar and 0.5% acetic acid. The fermentations were carried out as under the treatments summarised below (Table 1). During the fermentations carried out at 28°C for 45 days, acidity, reducing sugar and salt values were determined [Fleming *et al*., 1996].

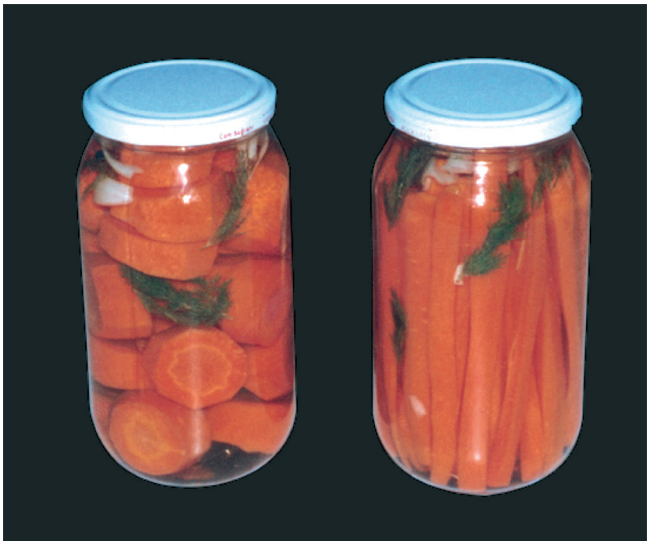

FIGURE 1. The slices placed in 1 L hermetic jars (1:1, w slices/v brine) with 1% onion, 0.1% black pepper and 0.5% dill.

TABLE 1. Sample coding of fermentation treatments applied to carrots.

Code	Treatment	Slice shape
C1	Spontaneous (Control 1)	Widthways
C2.1	2% yoghurt addition	Widthways
C2.2	2% whey addition	Widthways
C2.3	0.5% lactic acid addition	Widthways
C2.4	1% starter culture addition	Widthways
C3	Spontaneous (Control 2)	Lengthways
C4.1	2% yoghurt addition	Lengthways
C4.2	2% whey addition	Lengthways
C4.3	0.5% lactic acid addition	Lengthways
C4.4	1% starter culture addition	Lengthways

The carrots used had an acidity of 0.09% (as lactic acid). The acidity of yoghurt was 1.53% (L.A.), whereas in whey it was 0.11% (L.A.). The starter culture was a mix of equal amounts of *Lactobacillus plantarum, Leuconostoc mesenteroides, Lactobacillus brevis* and *Pediococcus cereviseae* (obtained from Uludag University, Faculty of Agriculture, Department of Food Engineering).

Sensory evaluation. The sensory characteristics of fermented carrots were judged by ten panellists after preliminary training sessions to instruct the panel according to the modified method described by Altug [1993] and Llorca *et al*. [2001], using sensory rating scale 1 to 5 (unacceptable/excellent). Panellists evaluated texture, colour, odour, taste and overall acceptability of pickled carrots throughout fermentation.

Statistical analysis. Analysis of variance (ANOVA), using 99% confidence intervals, was run on each of the physicochemical and sensory variable to disclose possible differences among the samples for the two factors "treatment" and "fermentation time". All analyses were performed using the Minitab for Windows (Version 10) Statistical Software Package (Minitab Inc., State College, PA).

TABLE 2. Effects of treatment and fermentation time on the chemical properties of pickled carrots.

Treatment code	N	Titratable acidity (%)	Salt (%)
C1	18	0.77 ± 0.006^{i}	3.13 ± 0.010^{f}
C2.1	18	0.85 ± 0.016^{ef}	3.28 ± 0.026^{d}
C2.2	18	0.80 ± 0.016^{h}	3.37 ± 0.023^{a}
C2.3	18	1.04 ± 0.013^{a}	3.07 ± 0.003^{g}
C2.4	18	0.89 ± 0.006^{d}	3.13 ± 0.020^{f}
C3	18	0.83 ± 0.023^{g}	3.27 ± 0.010^{d}
C4.1	18	0.86 ± 0.003^{e}	3.31 ± 0.020^{c}
C4.2	18	0.84 ± 0.013^{fg}	3.33 ± 0.010^{b}
C4.3	18	1.01 ± 0.003^{b}	3.14 ± 0.046^{f}
C4.4	18	0.92 ± 0.033^{c}	3.22 ± 0.023^{e}
Fermentation time (days)*			
1	20	0.43 ± 0.000^{g}	3.69 ± 0.028^{a}
3	20	0.38 ± 0.025^{h}	3.49 ± 0.012^{b}
6	20	0.54 ± 0.012^{f}	3.35 ± 0.015^{c}
8	20	0.65 ± 0.015^{e}	3.24 ± 0.012^{d}
10	20	0.83 ± 0.009^{d}	3.15 ± 0.015^{e}
13	20	0.97 ± 0.022^{c}	3.04 ± 0.006^{f}
15	20	1.40 ± 0.031^{a}	$3.02\pm.000^{g}$
30	20	1.37 ± 0.037^{b}	3.02 ± 0.012^{g}
45	20	1.37 ± 0.016^{b}	3.02 ± 0.022^{g}
ANOVA			
Treatment (T)		**	**
Fermentation Time (FT)		**	**
TxFT		**	**

Values are means of all fermentation time±standard error; different superscript letters after values indicate significant differences; Duncan's multiple range test ($p<0.01$,**). * The beginning of fermentation was taken as the first day of the fermentation period.

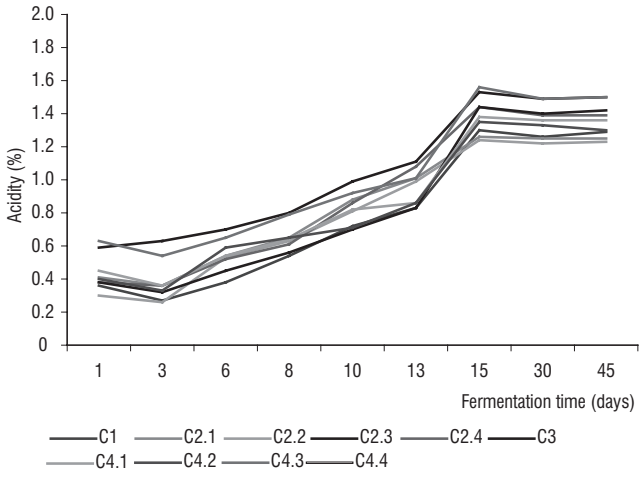

FIGURE 2. Changes in acidity of each treatment throughout 45 days of fermentation.

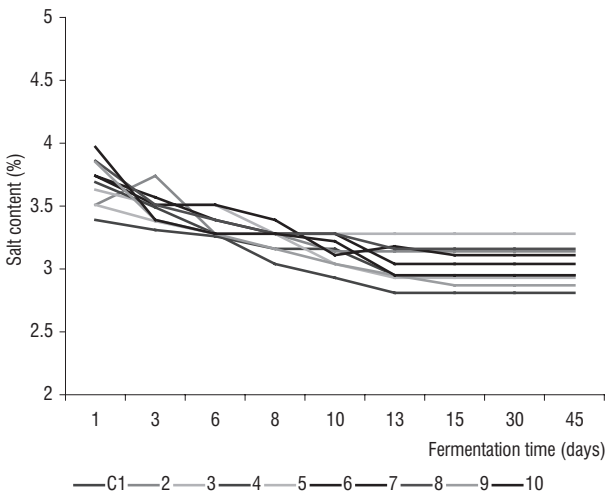

FIGURE 3. Changes in salt contents of each treatment throughout 45 days of fermentation.

RESULTS AND DISCUSSION

Fermentation process

Following brining an onset of material exchange started between brine and carrots. Fermentable sugar and other nutrients diffused in the brine supporting the activation of lactic acid bacteria leading to an increase in acidity as fermentation advanced up to the 15th day, after which the acidity of the brine decreased. Acidity did not varied significantly on the 30th and 45th day resembling the end of biochemical fermentation (Figure 2).

The salt contents of each treatment decreased from the 3rd day of fermentation due to material exchange and were balanced on the 15th day of fermentation (Figure 3).

The effect of treatments and fermentation time were significant on acidity and salt contents ($p<0.01$, Table 2). As designated, acidity increased as fermentation advanced, whereas salt contents declined. ANOVA test for acidity was between 0.77 and 1.04%, and salt contents were between 3.07 and 3.37%. The addition of acid resulted in high acidity and low salt intake. Acidity of samples C2.3 and C2.9 was slightly higher than that of treatments without added acid, whereas the lowest acidity was determined in samples C2.2 and C4.2. The salt intakes of pickles were *vice versa*. High acidity may reflect the activity of lactic acid bacteria supplemented with added lactic acid. The low acidity of whey treatment may be related to initial low acidity of the whey used.

Pickling of vegetables depends upon reduction of the activity of the enzymes responsible for chemical changes involved in fermentation and the inhibition of growth of dete-

TABLE 3. Effects of treatment and fermentation time on the sensory properties of pickled carrot.

Treatment code	N	Texture	Colour	Odour	Taste	Overall acceptability
C1	6	4.33 ± 0.017^{c}	4.33 ± 0.037^{c}	4.67 ± 0.057^{b}	4.67 ± 0.046^{c}	18.00 ± 0.040^{e}
C2.1	6	4.33 ± 0.011^{c}	5.00 ± 0.046^{a}	5.00 ± 0.323^{a}	4.67 ± 0.051^{c}	19.00 ± 0.005^{c}
C2.2	6	4.00 ± 0.577^{d}	5.00 ± 0.023^{a}	5.00 ± 0.127^{a}	5.00 ± 0.046^{a}	19.00 ± 0.577^{c}
C2.3	6	4.67 ± 0.040^{b}	5.00 ± 0.565^{a}	5.00 ± 0.357^{a}	4.67 ± 0.069^{c}	19.33 ± 0.017^{b}
C2.4	6	5.00 ± 0.028^{a}	5.00 ± 0.479^{a}	5.00 ± 0.098^{a}	4.67 ± 0.155^{c}	19.67 ± 0.155^{a}
C3	6	4.67 ± 0.028^{b}	4.67 ± 0.040^{b}	4.67 ± 0.028^{b}	4.67 ± 0.080^{c}	18.67 ± 0.098^{d}
C4.1	6	4.33 ± 0.023^{c}	5.00 ± 0.433^{a}	5.00 ± 0.011^{a}	4.83 ± 0.017^{b}	19.33 ± 0.011^{b}
C4.2	6	4.67 ± 0.034^{b}	5.00 ± 0.369^{a}	5.00 ± 0.069^{a}	5.00 ± 0.057^{a}	19.67 ± 0.037^{a}
C4.3	6	5.00 ± 0.011^{a}	5.00 ± 0.346^{a}	5.00 ± 0.147^{a}	4.67 ± 0.092^{c}	19.67 ± 0.086^{a}
C4.4	6	5.00 ± 0.577^{a}	4.67 ± 0.000^{b}	5.00 ± 0.254^{a}	4.33 ± 0.092^{d}	19.00 ± 0.000^{c}
Fermentation time (days)						
15	20	4.70 ± 0.031^{a}	5.00 ± 0.006^{a}	5.00 ± 0.053^{a}	4.85 ± 0.041^{a}	19.60 ± 0.037^{a}
30	20	4.60 ± 0.015^{b}	4.90 ± 0.025^{b}	5.00 ± 0.069^{a}	4.70 ± 0.063^{b}	19.20 ± 0.015^{b}
45	20	4.50 ± 0.047^{c}	4.70 ± 0.028^{c}	4.80 ± 0.056^{b}	4.60 ± 0.003^{c}	18.60 ± 0.015^{c}
ANOVA						
Treatment (T)		**	**	**	**	**
Fermentation Time (FT)		**	**	**	**	**
TxFT		**	**	**	**	**

Values are means of all fermentation time±standard error; different superscript letters after values indicate significant differences; Duncan's multiple range test ($p<0.01$,**). * The beginning of fermentation was taken as the first day of the fermentation period.

riorative microorganisms. Therefore, acid formation or addition is essential in conjunction with the salt to inhibit the softening due to enzymatic activity. Salt concentration is a key factor in fermentation process and final product quality [Luh & Woodroof, 1982; McDonald *et al*., 1991; McFeeters *et al*., 1995; Nabais *et al*., 1996]. Ballesteros *et al*. [1999] observed that the addition of a suitable starter shortened the fermentation process if the salt concentration in brine did not exceed 6%, compared to spontaneous fermentation.

Table 3 presents the mean values of sensory attributes in pickled carrot samples during 45 days of storage. Even though the ratings of sensory parameters did not range within wide limits, they were significantly affected by fermentation time. Texture, colour, odour, taste and overall acceptability ratings were the highest in all samples at the 15th day of fermentation, and decreased till the 45th day. Samples C2.4, C4.2 and C4.3 rated for the highest preference, whereas control samples for lengthways and widthways slices got the lowest points.

Final product

Acidity and salt contents of pickled carrots after 45 days of fermentation were presented in Figure 4. The acid values varied from 0.90 to 1.17%. Salt intake was the highest in control groups. As the result of withdrawal of water and nutrients from the vegetable tissue by salt, reducing sugar content of carrots tended to decrease, in comparison to raw material, as fermentation advanced. These nutrients furnish the substrate for growth and activity of the lactic acid bacteria. Nabais *et al.* [1996] described the diffusion of acid and salt into carrot slices as constant diffusivity, whereas the diffusion of reducing matter out of slices displayed a sigmoidal variation. This sigmoidal variation was explained by the assumption of proportional bursting of tiles to intact and burst cells.

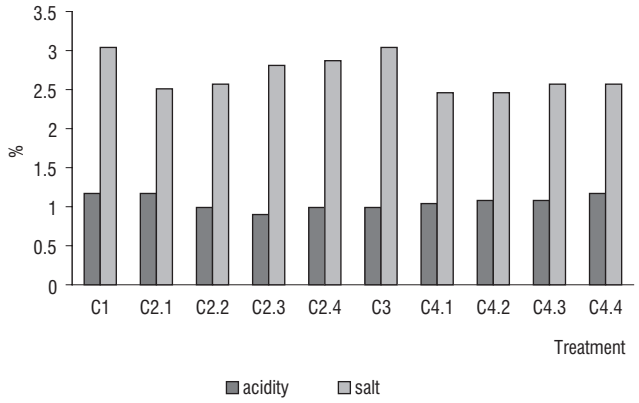

FIGURE 4. Acidity and salt contents of pickled carrots after 45 days of fermentation.

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