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Effect of a Pediococcus Culture on the Sensory Properties and Ripening of Anchovy Marinade at 4°C and 16°C

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Abstract

The effect of *Pediococcus* sp. 13 strain on the sensory properties and ripening of anchovy marinade at 4°C and 16°C was studied. Anchovies were ripened in 2% acetic acid and 10% NaCl solution at 4°C (group A), and at 16°C (group B). Inoculated samples were marinated similarly at 4°C (group C), and at 16°C (group D). pH values of all groups were below 4.5 and the mesophilic and halophilic bacterial counts were generally 2-3 log cfu/g during the study showing the safety of products. Appearance and taste of group D samples were significantly better ($P<0.05$) than others, and it was possible to consume them regarding their sensory properties after the 8th hour of marinating process. Even they were held at 4°C, appearance and taste of group C were acceptable for consumption similar ($P<0.05$) to group B (uninoculated at 16°C) after 16 h due to the inoculation with *Pediococcus* culture. Panelists considered group A samples proper for consumption at the 32nd h. at 4°C. It was concluded that marinated anchovies containing *Pediococcus* sp. 13 completed marination faster and obtained better sensory scores at either low (4°C) or ambient (16°C) temperatures.

Keywords: *Pediococcus*, anchovy, marinade, sensory quality, starter.

Pediococcus Kültürü İlavesinin 4°C ve 16°C'de Olgunlaştırılan Hamsi Marinatının Duyusal Özellikleri ve Olgunlaşması Üzerine Etkisi

Özet

Pediococcus sp. 13 suşunun 4°C ve 16°C'de hamsi marinatın duyusal özellikleri ve olgunlaşması üzerine etkisi araştırılmıştır. Hamsiler %2 asetik asit ve %10 NaCl içeren salamurada 4°C'de (A grubu) ve 16°C'de (B grubu) olgunlaştırılmıştır. *Pediococcus* sp. 13 inoküle edilen örnekler de 4°C'de (C grubu) ve 16°C'de (D grubu) marine edilmiştir. Tüm örnek gruplarında pH değerleri 4,5'in altında, mezofilik ve halofilik bakteri sayıları ise genel olarak 2-3 log kob/g düzeyinde bulunmuş olup, ürünün güvenli olduğunu göstermiştir. D grubu örneklerin görünüm ve tat puanları diğer gruplardan önemli düzeyde ($P<0.05$) daha yüksek olup, duyusal özelliklerine göre marinasyon işleminin 8. saatinden sonra tüketilebilir oldukları belirlenmiştir. Çalışmada, düşük sıcaklıkta marine edilmesine rağmen C grubu görünüm ve tat bakımından yüksek sıcaklıkta marine edilen B grubuna benzer şekilde ($P<0.05$) marinasyon işleminin 16. saatinden sonra tüketime uygun bulunmuştur. Bu durumda *Pediococcus* kültürü inoküle edilmesinin etkili olduğu düşünülmektedir. Panelistler 4°C'de marine edilen A grubu örnekleri 32. saatte tüketilebilir olarak değerlendirmişlerdir. Sonuç olarak, *Pediococcus* sp. 13 inoküle edilen hamsi marinat örnekleri gerek 4°C gerekse 16°C'de marinasyon işlemini daha hızlı tamamlamış ve daha yüksek duyusal puanları almıştır.

Anahtar Kelimeler: *Pediococcus*, hamsi, marinat, duyusal kalite, starter.

Introduction

Several methods could be used to protect fresh fish and seafoods such as cooking, cooling, smoking, drying, salting, canning and marinating (Sen and Temelli, 2003). Marination is one of the most popular types of these methods and it is obtained by treatment of fish with edible acids, usually acetic acid or vinegar, and salt. Acetic acid and salt give to the product its characteristic flavour and extend shelf life (McLay, 1972; Ludorf and Meyer, 1973; Gökoğlu *et al.*, 2004). Acetic acid decreases the pH of fish marinade to 4.3. Proteolytic enzymes are activated at this low pH, and degrade the proteins. Degradation

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products, i.e. amino acids, give the specific flavour of marinades. Ripening of marinades depends on fish: brine and salt: acid ratios, and temperature (Varlık *et al*., 1993). In Turkey, generally anchovy is used for commercial fish marinades (Gökoğlu *et al.*, 2004).

Lactic acid bacteria (LAB), known as GRAS, are used to increase sensory properties and quality of food. Although LAB are widely used as starter cultures for fermentation of milk, meat, cereal and vegetable products, they have not been utilized to any great extent in fish products except the fish sauces and silage fermentation (Gelman *et al.*, 2001). However, many studies have focused on use of LAB for fish products in recent years. Studies have been carried out to determine their performance as protective cultures (Jeppesen and Huss, 1993; Kim and Hearnsberger, 1994; Kim *et al.*, 1995a-b; Leroi *et al.*, 1996; Kışla and Ünlütürk, 2004) or as starter cultures for processing fermented fish products (Hwang *et al.*, 1989, Aryanta *et al.*, 1991; Morzel *et al.*, 1997a-b; Gelman *et al.*, 2001; Yin and Jiang, 2001; Asiedu and Sanni, 2002; Yin *et al.*, 2005). In this study, it was aimed to evaluate the effect of a *Pediococcus* strain on the sensory properties and ripening of anchovy marinade at 4°C and 16°C. Mesophilic and halophilic aerobic bacterial counts, and pH were also determined regarding the safety of anchovy marinades.

Materials and Methods

Anchovy (*Engraulis engrasicolus*) samples were purchased from wholesale fish market in Istanbul. Samples were transferred to laboratory in cooled conditions and used in the same day. The *Pediococcus* sp. 13 strain, which was originally isolated from fermented sausage (Cosansu *et al.*, 2007), was obtained from culture collection of Ankara University, Engineering Faculty, Department of Food Engineering.

Preparation of *Pediococcus* culture

Pediococcus sp. 13 was activated in De Man – Rogosa - Sharpe broth (MRS broth, Merck, Darmstadt, Germany), at 30°C for 24 h. The active culture was inoculated into MRS broth that contained NaCl at the concentration of 2% for adaptation to NaCl and incubated at 30°C for 24 h. The same procedure was repeated consecutively for 4% and 8% NaCl. A ten ml *Pediococcus* sp. 13 culture (10^6 CFU/ml) that grown in MRS broth supplemented with 8% NaCl was centrifuged at 3,354 *g* in a Centro mix centrifuge (Selecta, Barcelona, Spain) for 10 minutes at 4°C. And, then the supernatant was removed and the pellet was washed with sterile peptone water (0.1%). Following washing, the supernatant was discarded and the pellet was resuspended in 10 ml sterile peptone water (0.1%). For each 1 liter brine, a 10 ml-solution containing *Pediococcus* sp. 13 cells was used.

Preparation of Anchovy Marinade

The marination solution was prepared with acetic acid (2%) and NaCl (10%). Anchovies were marinated in glass jars. The fish: brine ratio was 1 : 1.5. Anchovies were headed, eviscerated, and filleted. Samples were divided into two groups and first group was separated as control and marinated in the brine without *Pediococcus* sp. 13 culture, and then stored at 4°C (group A) or 16°C (group B). The second group was treated in the marination solution containing *Pediococcus* sp. 13 culture. Following this procedure, samples were stored at 4°C (group C) or 16°C (group D).

Sensory Evaluation

Sensory evaluations of marinated anchovies were assessed on the basis of appearance, odour, and taste characteristics using a nine point descriptive scale (Amerina *et al.*, 1965; Gökoğlu *et al.*, 2004). Sensory evaluations were conducted using five trained and experienced panelists. The sensory panel consisted of people from the laboratory who were trained to identify ripening of fish marinade. Prior of this study, 10 people (6 female, 4 male, 25-40 years), who had been trained in sensory evaluation and had some experience with sensory tests for seafoods, were subjected to the sensory testing of different stages (raw anchovy, just after marinating process, ripened, aged in marinade solution, marketable quality) of anchovy marinade. This test was duplicated, and the best 5 perceivers were used as panelists in present study. Sensory panel members were not familiar with the treatment of the samples. To evaluate the ripening, the panellists were requested to give their opinion about acceptability of the samples as "fish marinade". Panellists evaluated the samples considering the typical appearance (texture and colour), taste (saltiness-sourness) and odour characteristics of anchovy marinade. The light and temperature conditions of laboratory, where the sensory analyses were carried out, were adjusted properly and panellists judged the samples in individual booths. A score of 7–9 indicated "very good" quality, a score of 4.0–6.9 "good" quality, a score of 1.0–3.9 denoted as "unacceptable".

Microbiological Analyses

Marinated anchovies were analyzed at 0, 8, 16, 24, 32 and 40th hours during marination. For microbiological analyses, samples of 10 g from each group were put in sterile plastic bags, mixed with 90 ml of peptone water (0.1%) and homogenized for 120 s at 50 Hz (IUL Masticator, Barcelona, Spain). Appropriate serial dilutions were made in 0.1% peptone water and plated by spreading 0.1 ml on duplicate plates of Plate Count Agar (PCA, Merck, Darmstadt, Germany) for total aerobic mesophilic and

Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) supplemented with 10% NaCl for total halophilic bacteria counts (Lakshmanan *et al.*, 2002). Lactic acid bacteria were enumerated by pour plating 1 ml of sample on MRS agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 30°C for 48 h. After incubation, the colonies were counted manually and counts were expressed as log CFU/g.

Chemical Analyses

The pH was measured on samples homogenized in distilled water (1/10 w/w) at room temperature. A WTW inoLab Level 1 pH Meter (Weilheim, Germany) was used for monitoring pH (Vyncke, 1981; Olafsdottir *et al.*, 1997).

For the determination of salt content (%, g NaCl in 100 g of fish sample), 5 g of the sample was homogenized and diluted using 250 ml of distilled water. This mixture was filtrated after heating in water bath for 1 h. Then, 25 ml of filtrate was mixed with the indicator (5% K_2CrO_4) and titrated with Ag NO_3 (0.1 N). The content of salt was calculated using the following formula (Varlık *et al.*, 2007):

$$\text{Salt [\%]} = \frac{0.00585 \times AgNO_3\ [\text{ml}]}{\text{Amount of the sample [g]}} \times 1\,000$$

Determination of total acidity was performed according to Varlık *et al.* (2007). Homogenized sample (20 g) was well mixed with 100 ml of distilled water and then filtrated. This filtrate was diluted to 250 ml with distilled water. A 20 ml of this mixture added indicator (1% phenolphthalein) was titrated using NaOH (0.1 N) and the content of total acidity was calculated as follows:

$$\text{Acidity [\%]} = \frac{0.6003 \times 12.5 \times \text{NaOH [ml]}}{\text{Amount of the sample [g]}}$$

Statistical Analyses

Statistical analyses were carried out according to Sümbüloğlu and Sümbüloğlu (2002) and ANOVA-test was performed using SPSS 11.0 (SPSS, Chicago, Illinois, USA). Differences between the treatments were evaluated ($P<0.05$) and Duncan's multiple range test was used.

Results

pH Values

The pH value should not be above 4.00-4.50 for marinated fish regarding products' safety. Acidic conditions make the tissue cathepsins much more active resulting in degradation of some muscle proteins into peptides and amino acids. These components give marinade its characteristic flavor and texture. In this study, pH values of all groups decreased significantly ($P<0.05$) at the first eight hours of marination, and then remained lower than 4.50, showing the safety of marinades for consumption (Table 1). Similarly, sharp decreases in pH values were observed in various studies on marinated anchovies (Şen and Temelli, 2003), sardine (Gökoğlu *et al.*, 2004; Kilinc and Cakli, 2004) and Pacific saury (Sallam *et al.*, 2007). Aksu *et al.* (1997) and Yapar (1998) marinated anchovies by immersing in a solution containing 2% acetic acid, and determined pH value as 4.50 and 4.25, respectively. Erkan *et al.* (2000) and Sallam *et al.* (2007) were also used 2% acetic acid for the marination of trout and Pacific saury, and estimated pH value as 4.33 and 4.37, respectively, whick is similar to our findings.

Kilinc and Cakli (2004) marinated sardines in a solution containing 7% acetic acid and reported the pH value as 3.80. Erdem *et al.* (2005) reported the pH value of anchovies marinated in 4% acetic acid as 4.11. These pH values are lower than those of our results due to the higher amounts of acetic acid. Likewise, Gökoğlu *et al.* (2004) reported a significant difference ($P<0.05$) between the pH values of fish marinated in 2% and 4% acetic acid solutions.

Salt and Acid Contents

Similar to the sharply decreased values of pH, acid concentrations also increased and determined as 0.63-0.67% after 8 hours of marination (Table 1). The minimum and maximum salt concentrations were 2.43-2.80% after 8 hours in marinade solution, and salt concentration was lower than 3.20 at the rest of marinating process (Table 1). Çelik (2004) marinated clams and reported their acid and salt concentrations as 0.68% and 2.93%, respectively. These results are harmonious with our findings.

Mesophilic and Halophilic Bacteria

It is known that the growth of bacteria has been suppressed by the acidic pH (Bjorkroth, 2005). Acetic acid, recognized as safe (GRAS), penetrates through the cell membrane of microorganisms and denature their proteins (Silliker *et al.*, 1980; Eklund, 1983). So, acidification is used for preservation of many foods, including fish (Sallam *et al.*, 2007). Salting has also been used for fish preservation. It inhibits microbial growth by restriction of the available water in food (Aubourg and Ugliano, 2002; Goulas and Kontominas, 2005). However, it is also known that bacteria are not completely killed by marination and are still viable in the semi-sterile medium (Fuselli *et al.*, 1994). In this study, the total mesophilic and halophilic bacteria counts were analyzed regarding the safety of marinated anchovies, and they were generally around 2–3 log CFU/g during the

Table 1. pH values, salt (%, g NaCl in 100 g of sample) and acid contents of marinated anchovies (mean±standard deviation)

Time [h]	pH			
	A	B	C	D
0	4.99±0.01[a]	5.10±0.00[b]	4.99±0.01[a]	5.10±0.00[b]
8	4.38±0.01[a]	4.33±0.00[b]	4.36±0.00[c]	4.35±0.00[d]
16	4.29±0.01[a]	4.34±0.02[b]	4.30±0.00[ac]	4.31±0.01[c]
24	4.35±0.01[a]	4.31±0.00[b]	4.42±0.00[c]	4.34±0.01[a]
32	4.27±0.01[a]	4.31±0.01[b]	4.35±0.01[c]	4.29±0.00[d]
40	4.29±0.01[a]	4.28±0.01[a]	4.29±0.02[a]	4.35±0.01[b]
	Salt (%)			
	A	B	C	D
0	2.18±0.02[a]	2.18±0.02[a]	1.66±0.15[b]	1.66±0.15[b]
8	2.80±0.05[a]	2.43±0.01[b]	2.53±0.08[c]	2.66±0.01[d]
16	2.60±0.08[a]	2.19±0.00[b]	2.30±0.05[c]	2.19±0.00[b]
24	2.57±0.01[a]	3.02±0.07[b]	2.72±0.05[c]	2.80±0.03[c]
32	2.80±0.10[a]	2.35±0.40[b]	2.60±0.10[c]	2.31±0.10[b]
40	3.19±0.09[a]	2.98±0.07[b]	3.00±0.02[b]	2.86±0.10[b]
	Total Acidity (%)			
	A	B	C	D
0	0.46±0.02[a]	0.46±0.02[a]	0.36±0.02[b]	0.36±0.02[b]
8	0.64±0.20[ac]	0.67±0.10[ab]	0.64±0.20[ac]	0.63±0.30[c]
16	0.56±0.04[a]	0.45±0.01[b]	0.53±0.02[a]	0.46±0.02[b]
24	0.56±0.04[a]	0.45±0.01[b]	0.53±0.02[a]	0.46±0.02[b]
32	0.73±0.03[a]	0.63±0.02[b]	0.66±0.02[b]	0.57±0.01[c]
40	0.65±0.02[a]	0.61±0.00[b]	0.61±0.01[b]	0.61±0.03[b]

A - uninoculated (4°C); B - uninoculated (16°C); C - inoculated (4°C), D - inoculated (16°C).
a, b, c - Different letters in the same row show the difference between the groups (P<0.05).

marination period (Table 2). Likewise, Sen and Temelli (2003) studied the microbiological and chemical qualities of marinated anchovy and determined total mesophilic aerobic bacteria count of anchovy marinades as 2.6 log CFU/g. The highest acceptable level for aerobic mesophilic bacterial counts of fish is 6 log cfu/g (ICMSF, 1986). In this study, the mesophilic aerobic bacterial counts did not exceed the recommended levels (Table 2).

Lactic Acid Bacteria

Marination process favours the growth of some LAB which is rarely detected in non-marinated foods. Acetic acid provides an acidic environment suitable for the action of proteolytic enzymes present in fish muscle. The products of proteolysis provide an energy source for the growth of LAB. Therefore many LAB species were detected in marinated products (Bjorkroth, 2005). Likewise, in present study LAB were detected in control samples (A and B), but their number did not reach to 2 log CFU/g during the study. Kilinc and Cakli (2004) reported the LAB count of sardine marinades as 1.30 log CFU/g which is similar to our findings.

As it was shown in Figure 1, *Pediococcus* sp. 13 inoculated samples (C and D) contained significantly higher (P<0.05) amounts of LAB than controls. However, C group samples, inoculated with LAB and stored at 4°C, contained lower (P<0.05) amount of LAB than D group samples during the study. That might be supported by Fuselli *et al.* (1994) and Lyhs (2002), who mentioned that low storage temperature may restrict growth of LAB in marinated fish.

Morzel *et al.* (1997a) studied on the influence of five strains of LAB on the quality of fermented salmon fillets. They proposed *L. sake* LAD and *L. alimentarius* BJ33 as suitable starters for fermentation of fish. In our study, *Pediococcus* sp. 13 isolated from fermented sausage was used and it was found as a potential culture to improve sensory properties of anchovy marinade. However, LAB strains isolated from fish and fish products might be more effective according to Gelman *et al.* (2001). Thus, to use a lactic culture isolated from a fish or fish product for marinated anchovies may present better results.

Sensory Properties

Sensory evaluation is the most popular way of assessing fish. It is fast, simple, and provides immediate quality information. The sensory characteristics of fish are clearly visible to the consumer and are essential for consumer satisfaction (Reineccius, 1990).

As it was shown in Figure 2, sensory scores of group A were generally lower (P<0.05), but the appearance and taste scores of group D were significantly higher (P<0.05) than those of the other groups at the 8th hour of this study. Group D ripened earlier than the others due to the higher temperature (16°C) and treatment with LAB, and they were accepted as "fish marinade" after 8 hours, and ripened according to panellists. It was also seen that, the sensory properties of group B and C were not significantly different (P>0.05) even they were

Table 2. Total aerobic mesophilic and halophilic bacteria counts (mean log CFU/g ± standard deviation)

Time	Mesophilic aerobic bacteria				Halophilic bacteria			
(h)	A	B	C	D	A	B	C	D
0	3.42±0.06^{a}	3.42±0.06^{a}	3.00±0.00^{b}	3.00±0.00^{b}	3.25±0.25^{a}	3.25±0.25^{a}	1.88±0.01^{b}	1.88±0.01^{b}
8	3.07±0.14^{ab}	2.69±0.30^{a}	3.00±0.09^{b}	2.82±0.11^{a}	2.83±0.20^{a}	3.20±0.04^{a}	2.00±0.00^{b}	1.69±0.22^{b}
16	2.51±0.05^{a}	3.02±0.07^{b}	3.54±0.11^{b}	3.23±0.04^{b}	3.80±0.16^{a}	3.15±0.15^{b}	3.25±0.00^{b}	3.11±0.09^{b}
24	2.24±0.09^{a}	3.04±0.00^{b}	3.15±0.00^{b}	4.30±0.00^{c}	2.00±0.00^{a}	2.15±0.21^{a}	2.17±0.00^{a}	2.32±0.21^{a}
32	2.00±0.00^{a}	2.30±0.07^{b}	3.00±0.00^{c}	3.47±0.00^{d}	1.69±0.01^{a}	2.00±0.14^{b}	1.69±0.03^{a}	2.00±0.07^{b}
40	2.54±0.03^{a}	3.39±0.06^{b}	1.69±0.01^{c}	2.58±0.01^{b}	2.30±0.42^{a}	1.69±0.00^{b}	1.69±0.00^{b}	2.00±0.00^{ab}

A - uninoculated (4°C); B - uninoculated (16°C); C - inoculated (4°C), D - inoculated (16°C).
a, b, c - Different letters in the same row show the difference between the groups (P<0.05).

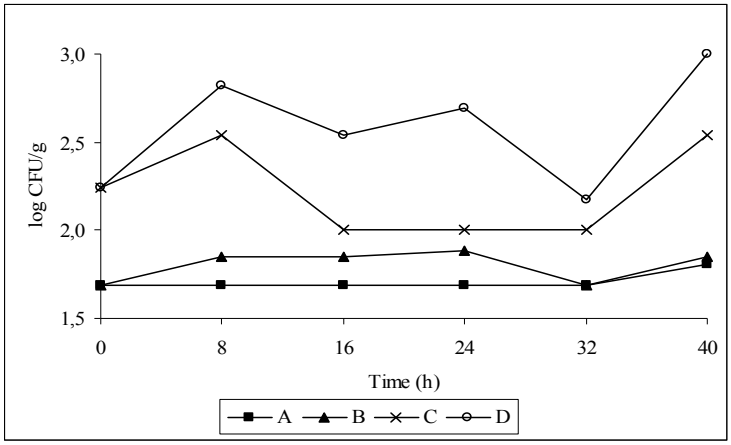

Figure 1. Lactic acid bacteria counts of anchovy marinades.
A - uninoculated, 4°C; B - uninoculated, 16°C; C - inoculated, 4°C; D - inoculated, 16°C.

allowed for marination at different temperatures (4°C and 16°C), and they were regarded as ripened after 16 hours. This might be the result of adding LAB to the samples stored at 4°C (group C). After 16 hours of marination, appearance and taste scores of group A were still lower (P<0.05) than those of the other groups. Their taste and appearance reached (P>0.05) to the level of other groups after 32 hours, and they ripened. Panellists did not mentioned significant difference (P>0.05) between the odour of samples during the study.

In marinated fish products, proteolytic enzymes are activated by acidic conditions, and degrade the proteins. Therefore, specific flavour of marinades is formed due to the degradation of proteins (Yapar, 1998). *Pediococcus* spp. produces proteolytic enzymes such as aminopeptidase, dipeptidase, protease, and dipeptidyl aminopeptidase (Bhowmik and Marth, 1990). On the other hand, Molly *et al.* (1997) mentioned both cathepsin D like muscle enzymes and bacterial enzymes play role in ripening and flavor generation during sausage fermentation. In our study, according to sensory analysis, group D (inoculated and marinated at 16°C) completed marination faster than group B (uninoculated and marinated at 16°C). Also, group C (inoculated and marinated at 4°C) completed this process faster than group A (uninoculated and marinated at 4°C). This means that the culture added groups were ripened faster than their controls. These differences may arise from the contribution of proteolytic enzymes produced by *Pediococcus* culture to ripening of marinade. Likewise, Morzel *et al.* (1997b) reported that LAB has positive effects on the sensory properties of fermented salmon fillets.

In this study, although *Pediococcus* sp. 13 has positive effect on the sensory properties, they have no significant effect on microbial and chemical properties of anchovy marinade. Likewise, *Pediococcus acidilactici* ST 79 and *Pediococcus pentosaceus* ST 13 were added as adjunct to Gouda cheese by Verachia (2005) and no significant effect was determined on the chemical composition or proteoliysis and lipolysis levels during the 45 days ripening period. However, sensory properties significantly (P<0.05) were affected by the addition of these cultures and more intense flavour was reported by the panelists for Gouda cheese prepared with *Pediococcus* species. Bhowmik and Marth (1990) mentioned that the use of Pediococci may provide advantage for ripening process of cheese. It is clear that, addition of *Pediococcus* species increases the sensory quality of the products.

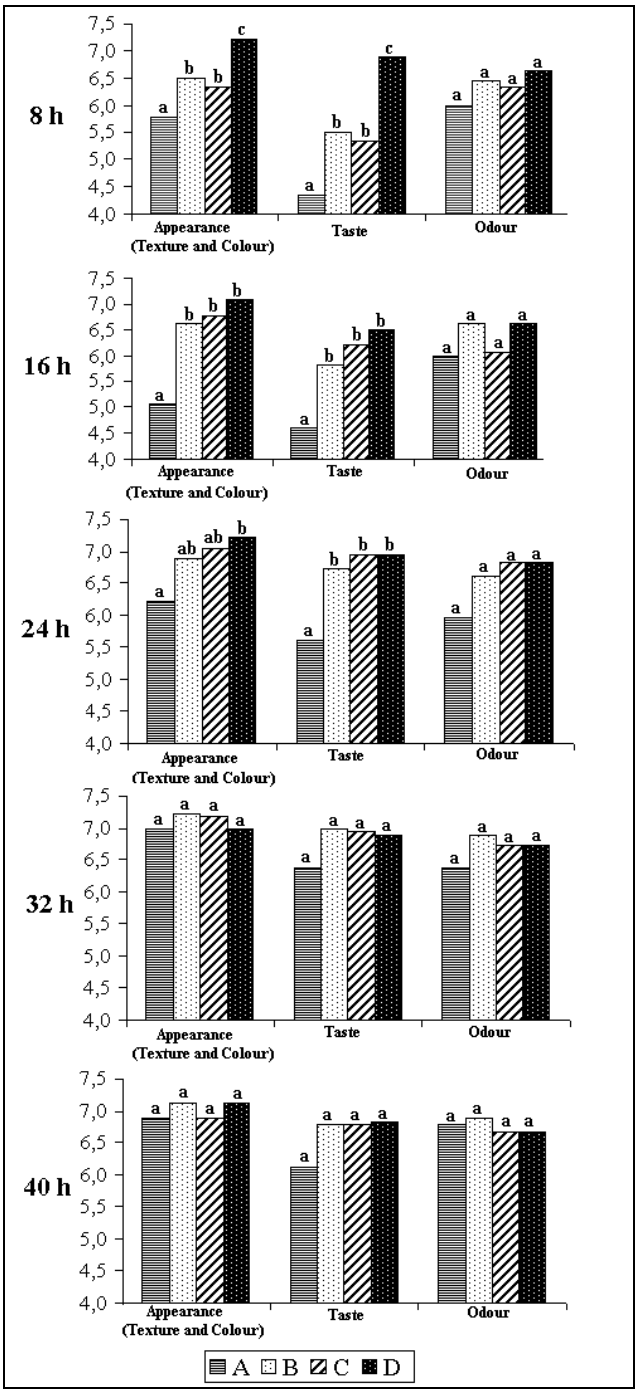

Figure 2. Sensory scores of marinated anchovy.
A - uninoculated, 4°C; B - uninoculated, 16°C; C - inoculated, 4°C; D - inoculated, 16°C.
a, b, c - Different letters above the bars show the difference between the groups (P<0.05)

Conclusion

In this study, low inoculation level of a *Pediococcus* sp. 13 was used to determine its effect on sensory properties and ripening of anchovy marinades. It was determined that marinated anchovies containing *Pediococcus* sp. 13, completed marination faster and obtained better sensory scores at either low or ambient temperatures. Considering the positive effects of this culture on sensory quality, higher inoculation levels of *Pediococcus* spp., especially originated from fish and fish products, may be studied for more remarkable results.

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