The effects of seasonal dynamics on sensory, chemical and microbiological quality parameters of vacuum-packed sardine (*Sardinella aurita*)

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Summary

The effects of seasonal changes (winter and spring) on the quality of sardines stored at 4 °C were investigated in terms of sensory, biochemical (thiobarbituric acid, total volatile basic nitrogen (TVB-N), peroxide value (PV) and free fatty acids (FFA) and microbiological parameters (total viable counts and coliforms). The limit for sensory acceptability of sardines stored in ice was 14 days and 18 days for winter and spring, respectively. Results showed that the TVB-N level did not exceed the acceptability limit (300–350 mg·kg^{-1}) in both winter and spring seasons. Significantly higher PV and FFA ($p < 0.05$) were determined for sardines in winter. Total viable counts of winter and spring sardines increased from the initial value of 3.40 log CFU·g^{-1} (day 0) to 7.74 log CFU·g^{-1} (day 18) and from 3.07 log CFU·g^{-1} to 6.33 log CFU·g^{-1} over the period of storage, respectively. In conclusion, samples obtained in winter season deteriorated more rapidly than those from the spring season. Chemical composition of fish, catching method, the condition of the fish before catching or harvesting, storage temperature and seasons may affect sensory, chemical and microbiological quality of fish.

Keywords:

sardine; quality; seasonal effect; chemical composition

Sardine is known to be a small pelagic fish species belonging to the genus *Clupeidae.* It is a commercially important fish species in Turkey being caught in large amounts (34 700 t in 2011) [1]. Sardine is rich in proteins, minerals, vitamins and polyunsaturated fatty acids (PUFA), mainly omega-3 fatty acids [2, 3]. These fatty acids are important in the prevention of human cardiovascular and inflammatory diseases [4–6]. However, nutritional composition and fatty acid composition of the fish are not constant. The chemical composition of fish muscle varies greatly within a species [7, 8] and between individuals depending on age, sex, environment and season [7, 9–11]. Actually, the variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning.

The chemical composition of different fish species shows variation depending on seasonal variation, migratory behaviour, sexual maturation, feeding cycles, etc. Fatty fish are particularly susceptible to lipid degradation, which can create severe quality problems even if stored at subzero temperatures [12]. Fresh fish is susceptible to spoilage caused by both microbial action and chemical reactions. Fish freshness is the most important and fundamental single criterion for judging the quality of fish and fishery products [13]. During storage, quality deterioration of fresh fish occurs rapidly and limits the shelf life of the product. The shelf life of fish in ice can be extended depending on raw material, the storage conditions (temperature and atmosphere), intrinsic factors such as species, age and size, fat content, feeding and physiological status, and the qualitative and quantitative composition of the initial microflora, related to the environment where the fish live and are caught, the seasonal period and the fishing method [14–17].

Vacuum-packaging (VP), along with refri-

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geration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution and marketing of raw and processed products to meet consumer demands. VP systems can provide further improvement in seafood shelf life, organoleptic quality and product range. Technologies such as VP have been shown to extend the shelf life of seafood products [18–24]. VP, in combination with refrigeration, has been proven to be an effective preservation method, in which the shelf life extension and quality retention of variety of fresh chilled food products e.g. red meat, poultry, fruits, vegetables, were achieved [25]. VP is widely used in the food industry because of its effectiveness in reducing oxidative reactions in the product at relatively low cost [26], extending the shelf life of seafood. The aim of this study was to determine the effects of the season on sensory, chemical and microbiological quality parameters of vacuum-packed sardine (*Sardinella aurita*) stored at 4 °C.

MATERIALS AND METHODS

Sardine (*Sardinella aurita*) was caught by net during 2010 winter and spring seasons in Mersin Bay, Turkey. The average weight of fish captured in winter was 34.03 ± 2.59 g and the length was 16.43 ± 0.61 cm. The water temperature was 13 °C and salinity was 4%. The average weight of fish captured in spring was 44.22 ± 3.55 g and the length was 16.83 ± 0.73 cm. The water temperature was 24 °C and salinity was 4%. Sardines were stored in boxes with ice when fish were on board after catching and during delivery to the laboratory. The fish were immediately filleted and divided into three groups. All groups were packaged in pouches of polyamide film (Polinas, Manisa, Turkey) using a vacuum packaging machine (Reepack RV50; Reepack, Seriate, Italy). The thickness of the film was 90 μm, water and oxygen permeability were 8.5 $g \cdot m^{-2}$ per 24 h and 160 $cm^3 \cdot m^{-2}$ per 24 h, respectively. Then, they were stored at 4 °C and, every 3 or 4 days throughout the storage time of 18 days, they were analysed sensorically, microbiologically and chemically.

Sensory analysis

The sensory evaluation of sardine fillets was done by the method of MENDES et al. [27]. The sensory evaluation of sardine fillets was conducted by a panel of 6–8 judges familiar with fish evaluation. Assessment was carried out for raw and cooked fillets (microwave cooking, 5 min at 100 °C), using a sensory scheme that evaluates fish quality by giving demerit points (d.p.) to each attribute evaluated. This scheme assigns scores of 0 d.p. to a maximum of 3 d.p. to each attribute, in which 0 represents high quality and higher score indicated lower fish quality. The scores of all attributes are summed to give an overall score (quality index, QI), that in the case of sardine fillets ranged from 0 d.p. to 12 d.p., the latter corresponding to fillets completely spoiled. It was considered that scores above 7.0 d.p. corresponded to unacceptable quality. Each panellist assessed one package (raw fillets) and one cooked fillet from each batch.

Proximate chemical analyses

Lipid content was measured by the method of BLIGH and DYER [28]. Ash and moisture contents were determined as described by AOAC method 920.153 [29] and AOAC method 950.46 [30], respectively. Protein was determined by the Kjeldahl procedure using a Büchi Digestion System, Model K-424 (Büchi Labortechnic, Flawil, Switzerland) and a Kjeltec Distillation Unit B-324 (Büchi Labortechnic). Percent protein was calculated as percentage of N × 6.25.

Analytical methods

Total volatile basic nitrogen (TVB-N) content of sardines was determined according to the method of ANTONOCOPOULOS [31] and expressed as milligrams TVB-N per kilogram of muscle. The value of thiobarbituric acid (TBA) was determined according to TARLADGIS et al. [32] in fish burger to evaluate the oxidation stability during storage period and the results expressed as TBA value, in milligrams of malondialdehyde (MA) per kilogram of flesh. Free fatty acids (FFA), expressed as percentage of oleic acid, were determined by AOCS method Ca 5a-40 [33]. Peroxide value (PV), expressed in milliequivalents of peroxide oxygen per kilogram of fat, was determined according to AOCS method Ja 8-87 [34].

Microbiological analyses

Samples from each of groups (triplicate) were taken to estimate total viable counts (TVC). Ten grams of fish were mixed with 90 ml of Ringer solution and then homogenized for 3 min. Further decimal dilutions were made up to 10^{-8} and 0.1 ml of each dilution was plated on count agar plates (cat. no. 70152; Fluka, Steinheim, Switzerland). Plates were prepared in triplicate and incubated for 2 days at 30 °C. For quantification of coliforms, Violet Red Bile Agar (cat. no. CM0107; Oxoid, Basingstoke, United Kingdom) was used, 1 ml aliquots of each dilution being applied using the

pour plate method and the plates were incubated for 24 h at 30 °C.

Statistical analysis

A completely randomized design was used. The data were subjected to analysis of variance and Duncan's multiple range tests using SPSS version 19 software (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Sensory assessment

Changes in the sensory quality of sardine fillets captured in winter and spring during refrigerated storage are shown in Tab. 1. According to the statistical analysis, no significant differences ($p > 0.05$) were found in the level of demerit points between winter and spring (except for 14th day) during the storage. The sensory scores for both winter and spring seasons increased regularly throughout the storage period. Sensory results of vacuum-packed sardines showed that the limits of acceptance of samples for winter were 14 days (8.42 d.p.) and 18 days (11.71 d.p.) for spring (Tab. 1). Other researchers have reported lower demerit scores for sensory limits of sardine [27, 35–37]. The sensory acceptability limit was 12 days for sardine stored in ice reported by Ozyurt et al. [38]. Chemical composition of fish, catching method, the condition of the fish before catching or harvesting, storage temperature and seasons were found to affect the results of sensory properties [39, 40].

Tab. 2 shows the results of the sensory assessment of cooked sardine fillets. The sensory score of the cooked fillets increased with storage time for both winter and spring seasons (11.75 d.p. and 11.29 d.p. at the end of storage period, respectively). Significant differences ($p > 0.05$) were ob-

Tab. 1. Sensory scheme for raw sardine fillets assessment.

Storage time [d]	Season	Skin appearance	Odour	Flesh firmness	Flesh colour	Total demerit score
0	Winter	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00
	Spring	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00
4	Winter	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00
	Spring	0.00 ± 0.00^{ax}	0.50 ± 0.00^{bx}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00
7	Winter	0.96 ± 0.11^{bx}	1.00 ± 0.00^{bx}	0.97 ± 0.08^{bx}	0.96 ± 0.11^{bx}	3.89
	Spring	1.00 ± 0.00^{bx}	1.00 ± 0.00^{cx}	0.91 ± 0.15^{bx}	1.07 ± 0.19^{bx}	3.98
11	Winter	1.43 ± 0.19^{cx}	1.49 ± 0.15^{cx}	1.57 ± 0.19^{cx}	1.60 ± 0.19^{cx}	6.09
	Spring	1.43 ± 0.19^{cx}	1.36 ± 0.24^{dx}	1.54 ± 0.11^{cx}	1.53 ± 0.08^{cx}	5.86
14	Winter	2.07 ± 0.19^{dy}	2.14 ± 0.24^{dy}	2.14 ± 0.24^{dx}	2.07 ± 0.19^{dx}	8.42
	Spring	1.51 ± 0.04^{cx}	1.54 ± 0.11^{ex}	2.00 ± 0.00^{dx}	1.93 ± 0.19^{dx}	6.98
18	Winter	3.00 ± 0.00^{ex}	2.93 ± 0.19^{ex}	2.93 ± 0.19^{ex}	2.86 ± 0.24^{ex}	11.72
	Spring	2.92 ± 0.19^{dx}	3.00 ± 0.00^{fx}	2.86 ± 0.24^{ex}	2.93 ± 0.19^{ex}	11.71

Values are expressed as mean ± standard deviation. a–f – indicate significant differences ($p < 0.05$) between seasons in a column ($n = 7$); x–y – indicate significant differences ($p < 0.05$) between seasons in a row.

Tab. 2. Sensory scheme for the assessment of cooked sardine fillets.

Storage time [d]	Season	Odour	Firmness	Succulence	Taste	Total score
0	Winter	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00
	Spring	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.00
4	Winter	0.00 ± 0.00^{ax}	0.56 ± 0.10^{by}	0.53 ± 0.08^{bx}	0.53 ± 0.08^{by}	1.62
	Spring	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}	0.50 ± 0.00^{bx}	0.00 ± 0.00^{ax}	0.50
7	Winter	0.56 ± 0.10^{bx}	0.60 ± 0.19^{bx}	0.87 ± 0.16^{cx}	0.93 ± 0.19^{cx}	2.96
	Spring	1.00 ± 0.00^{by}	1.07 ± 0.19^{by}	1.04 ± 0.11^{cy}	1.04 ± 0.11^{bx}	4.15
11	Winter	1.00 ± 0.00^{cx}	1.43 ± 0.19^{cx}	1.41 ± 0.19^{dx}	1.06 ± 0.10^{cx}	4.90
	Spring	1.50 ± 0.00^{cx}	1.93 ± 0.19^{cy}	1.86 ± 0.24^{dy}	1.07 ± 0.19^{bx}	6.36
14	Winter	2.14 ± 0.24^{dy}	2.14 ± 0.24^{dx}	2.07 ± 0.19^{ex}	2.14 ± 0.24^{dy}	8.49
	Spring	1.54 ± 0.11^{cx}	2.00 ± 0.00^{cx}	1.93 ± 0.19^{dx}	1.50 ± 0.00^{cx}	6.97
18	Winter	2.93 ± 0.19^{ex}	2.96 ± 0.11^{ey}	2.86 ± 0.24^{fx}	3.00 ± 0.00^{ex}	11.75
	Spring	2.86 ± 0.24^{dx}	2.71 ± 0.27^{dx}	2.79 ± 0.27^{ex}	2.93 ± 0.19^{dx}	11.29

Values are expressed as mean ± standard deviation. a–f – indicate significant differences ($p < 0.05$) between seasons in a column ($n = 7$); x–y – indicate significant differences ($p < 0.05$) between seasons in a row.

served in odour, firmness, succulence and taste after the 0th day of storage.

Chemical assessment

The moisture, crude protein, lipid and crude ash contents of sardine were found to be 77%, 20%, 1%, 1% and 74%, 20%, 5%, 1% in winter and spring, respectively. The proximate composition of the sardine showed similarities to the findings of ERKAN and OZDEN [36]. In other related studies, protein and lipid content in fresh sardine ranged from 16% and 5% to 20% and 12%, respectively [27, 35, 41]. BURT and HARDY [42] reported that the chemical composition of sardines was 57–78% water, 17–21% protein and 1–21% lipid.

TVB-N is known as a product of bacterial spoilage and endogenous enzymes action and its content is often used as an index to assess the keeping quality and shelf life of products [43]. TVB-N contents of sardine fillets (captured in winter and spring) stored at 4 °C are shown in Tab. 3. At the beginning of storage, TVB-N values were determined as 183.9 mg·kg^{-1} in winter samples and 162.5 mg·kg^{-1} in spring samples. Small differences in TVB-N values may be related to fish non-protein nitrogen content that depends on type of fish feeding, season of catching, fish size, various environmental factors as well as initial microbiological quality of the fish tissue [35]. TVB-N value increased in spring group until day 4, and then decreased to 158.1 mg·kg^{-1}. After that, this value increased regularly till the end of storage period. In sardines captured in winter, TVB-N value increased linearly throughout the storage period, reaching 294.5 mg·kg^{-1} at the end of the storage. Significantly ($p < 0.05$) lower initial TVB-N values were obtained for the spring group (162.5 mg·kg^{-1}) than for the winter group (183.9 mg·kg^{-1}). Considerable differences ($p < 0.05$) were observed between the winter and spring sardines during the storage period. LANG [44] suggested that the quality classification of fish and fish products regarding TVB-N values would be "high quality" when up to 250 mg·kg^{-1}, "good quality" when up to 300 mg·kg^{-1}, "limit of acceptability" when up to 350 mg·kg^{-1}, and "spoilt" when above 350 mg·kg^{-1}. In this study, the acceptability limits (350 mg·kg^{-1}) for TVB-N were not exceeded throughout storage for both winter and spring groups. As for many fish species, the formation of TVB-N increased with time of storage. When TVC had reached 10^6 CFU·g^{-1}, the TVB-N contents in muscle for winter and spring seasons were found to be approximately 246.4 mg·kg^{-1} and 202.5 mg·kg^{-1}, respectively. The more rapid increase of TVB-N at higher microbial numbers indicated the stage of substantial spoilage of the fish. TVB-N contents were correlated well with results of sensory analyses of sardines. TVB-N contents of sardines for both seasons increased with an increase in sensory scores.

Tab. 3. Content of total volatile basic nitrogen in sardines captured in winter and spring during the storage period.

Storage time [d]	TVB-N [mg kg^{-1}]	
	Winter	Spring
0	183.9 ± 0.35^{ay}	162.5 ± 0.42^{ax}
4	199.6 ± 0.43^{by}	166.9 ± 0.05^{ax}
7	213.8 ± 0.42^{cy}	158.1 ± 0.41^{ax}
11	246.4 ± 0.32^{dy}	216.2 ± 0.71^{cx}
14	266.9 ± 0.87^{ey}	208.7 ± 0.01^{bcx}
18	294.5 ± 0.39^{fy}	202.5 ± 0.73^{bx}

Values are expressed as mean ± standard deviation ($n = 7$). a–f – indicate significant differences ($p < 0.05$) between seasons in a column; x–y – indicate significant differences ($p < 0.05$) between seasons in a row.

A smilar observation was reported by ERKAN and OZDEN [36] for whole and gutted sardine (*Sardina pilchardus*) stored at 4 °C. They found TVB-N values to be 292.3 mg·kg^{-1} and 150.3 mg·kg^{-1} for whole and gutted sardines, respectively. MENDES et al. [27] observed this value as 181 mg·kg^{-1} on 12th day of storage. However, higher values were obtained by GOULAS and KONTOMINAS [45] in vacuum-packed sardines, 427 mg·kg^{-1} on 17th day of storage. Additionally, this value reached 554 mg·kg^{-1} at the end of the storage period (23th day). Similarly, KENAR et al. [46] reported the TVB-N values of sardine to be 462.8 mg·kg^{-1} on 17th day of storage period. In our study, the TVB-N values of sardines captured in winter were higher than those of the spring sardine flesh. The high initial contents of TVB-N may be attributed to the high level of non-protein nitrogen present in the flesh of sardine, which in turn depends on fish feeding type, catching season, fish size, age and locality [47]. The increase in TVB-N is related to the activity of spoilage bacteria and endogenous enzymes, storage conditions, hygienic practices, etc. [48].

TBA is a second breakdown product of lipid oxidation and is widely used as an indicator of the degree of lipid oxidation. The temperature, storage conditions and processing methods are known to affect the lipid oxidation degree [49]. Tab. 4 shows TBA contents during storage of sardines in winter and spring seasons. At the begin-

Tab. 4. Content of thiobarbituric acid in sardines captured in winter and spring during the storage period.

Storage time [d]	TBA [mg·kg^{-1}]	
	Winter	Spring
0	1.14 ± 0.11^{dy}	0.71 ± 0.08^{bx}
4	0.55 ± 0.02^{bcx}	0.57 ± 0.05^{ax}
7	0.50 ± 0.02^{abx}	0.74 ± 0.04^{by}
11	0.56 ± 0.04^{cx}	0.94 ± 0.13^{cy}
14	0.46 ± 0.02^{ax}	1.62 ± 0.22^{dy}
18	0.46 ± 0.01^{ax}	0.90 ± 0.07^{cy}

Values are expressed as mean ± standard deviation (n = 7). TBA values are expressed as milligrams of malondialdehyde. a–f – indicate significant differences ($p < 0.05$) between seasons in a column; x–y – indicate significant differences ($p < 0.05$) between seasons in a row.

ning of the storage, TBA values were determined as 1.14 mg·kg^{-1} and 0.71 mg·kg^{-1} for the sardine captured in winter and spring, respectively. Significantly ($p < 0.05$) higher TBA values were observed in spring season, reaching 1.62 mg·kg^{-1} on 14th day of storage. This could be due to the fact that spring samples had a higher lipid content than winter samples. Fish contain long-chain unsaturated fatty acids (PUFA), which are prone to oxidation. In both spring and winter seasons, this value showed fluctuations throughout storage and remained constant after the 14th day of storage in winter season (0.46 mg·kg^{-1}). Similar results were reported for sardine by KENAR et al. [46] and LOSADA et al. [50]. The content of TBA in freshly caught fish is typically between 3 mg and 5 mg of MA equivalents per kilogram flesh, but levels of 5–8 mg of MA equivalents per kilogram of flesh are generally regarded as the limit of acceptability for fish stored in ice [39, 51, 52].

The primary product of lipid oxidation is fatty acid hydroperoxide, measured as PV. Peroxides are unstable compounds, and they break down to aldehydes, ketones and alcohols that are volatile products causing off-flavour in products [53]. In this study, PV was employed for determining the formation of primary oxidation products during the storage period in sardine fillets. The initial peroxide values were determined as 11.27 meq·kg^{-1} and 2.71 meq·kg^{-1} for winter and spring seasons, respectively. Until the 11th day of storage, PV increased regularly in the winter season and then decreased during the rest of storage period, whereas this value increased in the spring season after 7th day of storage. PV was significantly greater in winter sardines and there were significant differences ($p < 0.05$) between winter and spring seasons during the storage period (Tab. 5). KENAR et al. [46] reported that, in sardines stored at 4 °C for 20 days, PV showed fluctuations throughout the storage, while this value increased regularly in whole and gutted sardines stored at 4 °C for 9 days during the storage period [36]. The lipid oxidation was attributed to the combination of free radicals with O_2 to form hydroperoxides. PV value below 5 meq·kg^{-1} indicates that fat is fresh or hydroperoxides have degraded into ketones. PV value between 5 meq·kg^{-1} and 10 meq·kg^{-1} indicates that fat is commencing rancidity [54].

It is well known that FFA are a result of enzymatic decomposition of lipids in frozen fish [55]. The initial level of FFA was 11% for winter sardines, reaching the maximum level of 20% at the end of storage (Tab. 6). Considerable differences ($p < 0.05$) were observed in FFA between the winter and spring seasons. A lower FFA levels were observed in spring season and fluctuations were

Tab. 5. Peroxid values of sardines captured in winter and spring during the storage period.

Storage time[d]	PV [meq·kg^{-1}]	
	Winter	Spring
0	11.27 ± 0.35^{ay}	2.71 ± 0.16^{ax}
4	19.52 ± 0.42^{by}	5.77 ± 0.21^{cx}
7	26.75 ± 0.26^{ey}	4.55 ± 0.76^{bx}
11	30.27 ± 0.19^{fy}	5.95 ± 0.33^{cx}
14	25.12 ± 0.14^{dy}	11.70 ± 0.42^{dx}
18	21.48 ± 0.87^{cy}	13.01 ± 0.59^{ex}

Values are expressed as mean ± standard deviation (n = 7). a–f – indicate significant differences ($p < 0.05$) between seasons in a column; x–y – indicate significant differences ($p < 0.05$) between seasons in a row.

Tab. 6. Content of free fatty acids in sardines captured in winter and spring during the storage period.

Storage time [d]	FFA [%]	
	Winter	Spring
0	11 ± 0^{aby}	3 ± 0^{ax}
4	8 ± 0^{ay}	3 ± 0^{ax}
7	12 ± 1^{by}	3 ± 0^{ax}
11	20 ± 2^{cy}	5 ± 0^{bx}
14	11 ± 0^{aby}	6 ± 0^{dx}
18	20 ± 2^{cy}	5 ± 0^{cx}

Values are expressed as mean ± standard deviation (n = 7). FFA values are expressed as percentage of oleic acid. a–f – indicate significant differences ($p < 0.05$) between seasons in a column; x–y – indicate significant differences ($p < 0.05$) between seasons in a row.

observed in both groups during the storage period (Tab. 6). FFA and their oxidation products would have an effect on muscle texture and functionality since they interact with myofibrillar proteins and promote protein aggregation [56]. In the present study, the release of FFA increased ($p < 0.05$), it was 20% (expressed as percent of oleic acid) for winter samples and 5% for spring samples at the end of the storage period (Tab. 6). Lipid hydrolysis developed at a slower rate in sardines captured in spring.

Similar results were reported in our previous research [46, 57]. An increase in FFA (lipolysis) results from the enzymatic hydrolysis of esterified lipids [58]. The connection between lipolysis and lipid oxidation rancidity is substantiated in the fact that free (polyunsaturated) fatty acids are oxidized more readily than esterified lipids [59].

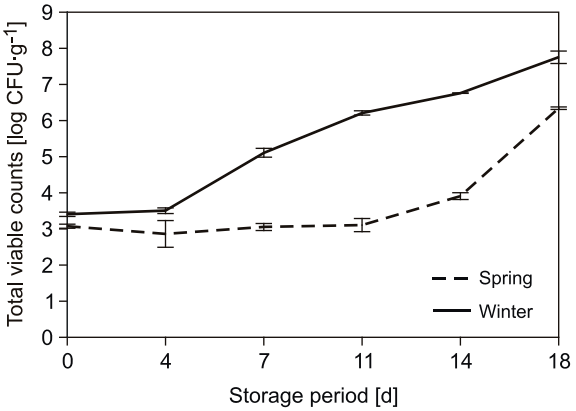

Fig. 1. Total viable counts of vacuum-packed sardines.

Microbiological assessment

Total viable counts are used as an acceptability index for fish products because of the role of bacteria in spoilage. These values for sardines during the storage period are presented in Fig. 1. Initial total viable counts of sardine were 3.40 log CFU·g^{-1} and 3.07 log CFU·g^{-1} captured in winter and spring, respectively. Significant differences ($p < 0.05$) were observed between winter and spring seasons. The microbiological condition of fish muscle is known to be directly related to fishing ground and environmental factors [60–64]. The method by which fish are harvested, on board handling, storage and transportation are also mentioned as influencing the numbers and types of bacteria on the raw material [65–68]. For fresh water and marine species, the microbiological limit recommended by ICMSF [69] for TVC at 30 °C is 7 log CFU·g^{-1}. TVC increased during the storage period and, in winter season, exceeded the limit (> 7 log CFU·g^{-1}) after 18th day of storage (Fig. 1). Microbiological results were in agreement with sensory results for fish caught in spring (shelf life of 18 days). However, sensory analysis results showed shorter shelf life (14 days) for fish caught in winter. This could have been due to higher bacterial contamination during harvesting and handling. Hygienic handling of the fish from the moment of catching is very important in order to ensure good quality and long storage life. Huss [12] also indicated that bacteria on fish caught in temperate waters will enter the exponential growth phase almost immediately after the fish have died. This is also true when the fish are iced, probably because the microflora is already adapted to lower temperatures.

We reported that initial TVC of sardines with a 2 days post-capture history was 3.8 log CFU·g^{-1}, and that the limit of acceptability was reached at day 3 for sardines stored in air, at day 8 in vacuum and after 10 days in modified atmosphere packing (MAP) (60% CO_2, 40% N_2) [31]. In sardines with a 3 days post-capture history, an initial TVC of 3.8 log CFU·g^{-1} was also reported, with the limit of acceptability of 6 days for air-packed fillets, 13 days for vacuum-packed and more than 15 days for MAP fillets (50% CO_2, 50% N_2) [70]. The authors also reported that the initial TVC (3.8 log CFU·g^{-1}) indicated good fish quality. It was reported that increased CO_2 content in the packing atmosphere increasingly inhibited the growth of spoilage bacteria [71] and increased the shelf life of fishery products [72]. Microbiological results in this study showed a better quality than the findings of other authors in relation to the usefulness of vacuum packing [35, 70].

During the storage period total coliforms and *E.coli* were not observed in sardines captured in both winter and spring seasons (data not shown). Mersin Bay has not been exposed to fecal contamination since coliforms and *E.coli* were not reported in sardines captured in this location.

CONCLUSION

Results of this study showed that significant differences in sensory, chemical and microbiological parameters ($p < 0.05$) were observed between sardines captured in winter season and spring season throughout the storage period at 4 °C. Sensory results of vacuum-packed sardines were within the limits of acceptance for 14 days in the winter season (8.42 d.p.) and 18 days (11.71 d.p.) in the spring season. The formation of TVB-N

increased with time of storage for both seasons. However, the acceptability limits (350 $mg \cdot kg^{-1}$) for TVB-N were not exceeded throughout storage for both groups. Significantly ($p < 0.05$) higher TBA values were observed in spring season and reached the maximum level of 1.62 $mg \cdot kg^{-1}$ at 14th day of storage. PV was significantly greater in the winter sardines than in spring sardines. Lower FFA was observed for spring and showed fluctuations in both groups during the storage period. TVC increased during storage. According to data obtained from this study, samples obtained from winter season deteriorated more rapidly than those from spring season. Chemical composition of fish, catching method, the condition of the fish before catching or harvesting, storage temperature and season may affect sensory, chemical and microbiological quality of fish. Hygienic handling of the fish from the moment of catching is also very important in order to ensure good quality and long storage life.

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