THE EFFECTS OF SEASONING ON THE QUALITY OF PRECOOKED AND VACUUMED CRAB MEAT (*PORTUNUS PELAGICUS*)

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ABSTRACT

The effects of seasoning with herb leaves (rosemary, oregano and laurel) on the quality of precooked (83 °C for 10 min) and vacuumed crab meat were evaluated in terms of microbiological, sensory and chemical analyses. According to sensory results, the limit for sensory acceptability of precooked and vacuumed crab meat stored at 4 °C was ~38-40 days for the control whereas the shelf-life of the samples with herb leaves were ~ 40-42 days. As spoilage progressed, the off-flavour increased in intensity until the crab meats were no longer edible at 42 days. The TVB-N values for the treated groups remained low compared to those of the control. The lowest TVB-N value was obtained from the sample with thymus leaves ($p<0.05$) whereas the control group gave the highest TVB-N value ($p<0.05$) at 38 days. TVB-N values did not correlate with sensory analysis, since the level of TVB-N content ranged from 35.41 mg/100 g for the group with laurel to 89.21 mg 100 g^{-1} for the control group at the time of rejection, regarding to be "spoilt" above 35 mg 100 g^{-1} for fish and fish products. TBA values for all groups were found to be quite low during the storage period. According to microbiological analyses (10^7 microorganisms/g are considered), the TVC limit of acceptability, the shelf-life of precooked and vacuumed crab meat were approximately ~38-40 days for the control and ~42-44 days for treated groups, indicating that sensory analysis of crab meat correlated well with microbiological analyses. Generally, seasoning with herb leaves improved the sensory quality of precooked and vacuumed crab meat, also delaying its spoilage.

KEYWORDS:
crab meat, *Portunus pelagicus*, seasoning, shelf life, quality

1. INTRODUCTION

Crabs are a large group of invertebrates, and commercial fisheries have focused on crabs since they have not only delicious taste, but also a high palatability and unique pleasant aroma [1]. Swimming crabs (*Portunus pelagicus*) annual output has increased during the past decade in Turkey, from 55,071 tons in 2003 to 95,871 tons in 2007 [2]. The crab industry is generally interested in pasteurisation, freezing and canning. Therefore, the quality of raw material is considered to be the most important factor, determining the quality and safety of the end product [3].

Seafood products, including crustacean shellfish, have been paid attention for their health-promoting characteristics. It is well-documented that polyunsaturated fatty acid (PUFA) contents are beneficial in the reduction of heart diseases [4-9]. Although the nutritional composition of several commercially important species of crabs has been characterized, they vary widely in their nutrient content [10-12]. Kuley *et al.* [13] also reported differences in concentrations of moisture, fat, ash, protein, and in meats from different sex and body parts of the blue crab. The composition of proteins, amino acids, minerals, sterols, total lipids and fatty acids of the most commercially significant crabs can be found in previous publications [11, 14-19].

Microbial spoilage is the primary factor in quality deterioration of fresh meats, whereas lipid oxidation is another critical factor in the spoilage of meats. Ready-to-cook (RTC) and ready-to-eat (RTE) products are the principle means in order to control spoilage and foodborne pathogens in these types of foods [20]. RTE products are less likely to cause foodborne illness than RTC products due to heat treatment during cooking, thus reducing or eliminating all foodborne pathogens [21]. Most of the plant extracts examined (e.g. bay leaf, marjoram, or thyme) are commonly used as cooking herbs. The application of these herbs to RTC and RTE products can improve food safety and enhance flavour [22].

Herbs are leafy, green plant parts used for purposes of flavour, colour, or as a preservative that prevents growth of harmful bacteria. The leafy parts of plants, such as oregano, thyme and savory, belonging to the Lamiaceae family, have been added to meat, fish and food products

for many years [23]. The antioxidant activity of herbs and spices, caused mainly by phenolic compounds, has been demonstrated in many studies. The main compounds responsible for the high antioxidant effect of herbs are the concentrations of phenol compounds, such as carnosic acid, carnosol and rosmarinic acid [24], among them hyssop (*Hyssopus officinalis* L.), rosemary (*Rosmarinus offici*nalis L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and clove (*Sysygium aromaticum* L.) [25, 26], and bay laurel (*Laurus nobilis* L.) [27].

A research is currently underway to investigate the feasibility of developing and marketing precooked crab products treated with different herb leaves for European markets. The principal objectives of this investigation were: (i) to determine the shelf-life of the product treated with various herb leaves; (ii) to evaluate some of the existing objective tests as indices of quality and degree of spoilage of precooked crab meat; and (iii) to determine antioxidant effects of herbs on precooked and vacuumed crab meat.

2. MATERIALS AND METHODS

2.1. Sample preparation

The crabs were caught by a dip net in the eastern Mediterranean Sea and transported to laboratory alive. Average weight, carapace width and carapace length of the crabs were 210.1±30.6 g, 145±0.6 mm and 77.1±0.3 mm, respectively. Carapaces of both raw specimens were removed, and the two largest portions of meat connected to the swimming legs were carefully scraped out with a scalpel. Crab meat was precooked at 83 ^{o}C for 10 min, and then divided into 4 groups. In this project, thymus, rosemary and laurel herbs were dried and chopped into smaller pieces. Each herb (%0.6) was added to crab meats, and then all groups were vacuum-packaged in polyethylene bags. The samples were stored under refrigerator conditions (4±1 ^{o}C). The control group did not contain any spice. Each polythene bag contained approximately 0.5 kg sample.

2.2. Sensory analysis

Sensory analysis (appearance, odour, flavour and texture) of crab meats treated with rosemary, thymus and laurel spices were assessed according to the method of Paulus *et al.* [28]. A 9-point hedonic scale was used to evaluate crab meats treated with spices. A score of 9 represents 'very good quality', a score of 7-8 'good quality', a score of 5-6 'acceptable', while a score of 1-4 was regarded to be 'bad or unacceptable'.

2.3. Analytical methods

The TVB-N content of crab meat was determined according to the method of Antonacopoulos [29], and expressed as mg TVB-N per 100 g muscle. The value of TBA was determined according to Tarladgis *et al*. [30] in crab meat to evaluate the oxidation stability during storage period, and the results were expressed as TBA value (mg of malondialdehyde per kg flesh). Biogenic amines were analysed using an HPLC method [31]. Benzoyl chloride as a derivatization reagent was used, and derivatization procedure was based on that of Redmond and Tseng [32].

2.4. Apparatus

For High-performance liquid chromatography (HPLC) analyses, a Shimadzu LC-10VP (Shimadzu, Kyoto, Japan) apparatus was used, equipped with a UV/VIS detector (Spectra-Physics SP 8450, Analytical Inc., UK) and a low gradient pump (Shimadzu LC-10ATVP) with 4-channel mixer (Shimadzu FVC-10ALVP). For biogenic amine analysis, the column used was a reversed-phase Nucleosil C18 (250x4.6 mm, particle diameter 5 µm; Macherey-Nagel, Duren, Germany).

2.5. Microbiological analyses

Samples from each group (triplicate) were taken to estimate total viable counts (TVC). Ten g of crab meat were mixed with 90 ml of Ringer solution, and then homogenised for 3 min. Further decimal dilutions were made up to 10^{-8}, and then 0.1 ml of each dilution was pipetted onto the surface of a plate count agar plate, in triplicate.

Total plate counts of herbs obtained from a crab processing plant were also determined. They were placed aseptically in sterile bottles, and transported in ice to a laboratory where plate counts were made immediately. One gram of each herb was mixed with 9 ml of Ringer solution; the samples were further diluted and plated on plate count agar medium. Then, all plates were prepared in triplicate and incubated for 2 days at 30 ^{o}C.

2.6. Statistical analyses

A completely randomized design was used. The data were subjected to analysis of variance and Duncan's multiple range tests (SPSS statistical package, version 8.0, adapted to a PC).

3. RESULTS

3.1. Sensory assessment

Tables 1-3 show the results of sensory assessment of precooked and vacuumed crab meat treated with herbs. The off-flavour intensity of the treatment groups remained at low levels, compared to the flavour of herbs in control group, until the end of the storage period.

3.2. Chemical assessment

TVB-N concentrations of the precooked and vacuumed crab meat treated with herbs leaves are shown in Table 4. At the beginning of storage, the TVB-N value was in the range of 25.67-27.56 mg/100 g flesh for the samples. The TVB-N values showed an increasing pattern during stor-

TABLE 1 - Initial sensory assessment (day 1) of precooked and vacuumed crab meat treated with herbs leaves.

Day 1	Control $\overline{X} \pm S_{\overline{X}}$	Thymus $\overline{X} \pm S_{\overline{X}}$	Rose-mary $\overline{X} \pm S_{\overline{X}}$	Laurel $\overline{X} \pm S_{\overline{X}}$
Appearance	8.63±0.52^{a}	8.63±0.52^{a}	8.63±0.52^{a}	8.38±0.52^{a}
Odour	7.88±0.35^{a}	8.88±0.35^{c}	8.00±0.00^{a}	8.50±0.54^{b}
Texture	8.38±0.52^{a}	8.50±0.76^{a}	8.50±0.54^{a}	8.38±1.06^{a}
Colour	7.88±0.99^{a}	8.75±0.46^{b}	8.13±0.35^{ab}	8.63±0.52^{b}
Taste	7.38±1.19^{a}	7.63±1.30^{a}	8.25±0.46^{a}	7.50±2.00^{a}
General Acceptance	8.00±0.76^{a}	8.50±0.76^{a}	8.13±0.35^{a}	8.25±0.71^{a}

* Different letters in the same row indicate significant differences ($p<0.05$); $\overline{X} \pm S_{\overline{X}}$: average± standard deviation

TABLE 2 - Sensory assessment (Day 38) of precooked and vacuumed crab meat treated with herb leaves.

Day 38	Control $\overline{X} \pm S_{\overline{X}}$	Thymus $\overline{X} \pm S_{\overline{X}}$	Rosemary $\overline{X} \pm S_{\overline{X}}$	Laurel $\overline{X} \pm S_{\overline{X}}$
Appearance	7.13±0.99^{a}	7.50±0.76^{a}	7.25±0.89^{a}	7.25±0.89^{a}
Odour	6.25±1.17^{a}	7.25±0.46^{a}	7.50±1.41^{a}	7.38±1.06^{a}
Texture	5.25±0.71^{a}	6.13±0.84^{a}	5.63±0.74^{a}	5.88±1.36^{a}
Colour	6.00±1.51^{a}	6.50±1.31^{a}	6.13±1.46^{a}	6.13±1.81^{a}
Taste	6.38±1.06^{b}	7.50±1.85^{a}	7.15±1.49^{a}	7.50±1.31^{a}
General Acceptance	6.24±1.41^{a}	6.88±1.89^{b}	6.63±1.41^{b}	6.75±1.05^{b}

* Different letters in the same row indicate significant differences ($p<0.05$); $\overline{X} \pm S_{\overline{X}}$: average ± standard deviation

TABLE 3 - Sensory assessment (Day 42) of precooked and vacuumed crab meat treated with herbs.

Day 42	Control $\overline{X} \pm S_{\overline{X}}$	Thymus $\overline{X} \pm S_{\overline{X}}$	Rosemary $\overline{X} \pm S_{\overline{X}}$	Laurel $\overline{X} \pm S_{\overline{X}}$
Appearance	4.00±0.54^{a}	5.38±0.74^{b}	3.13±1.36^{a}	5.50±0.76^{b}
Odour	3.00±0.54^{a}	4.75±0.71^{b}	3.75±1.04^{a}	5.00±1.07^{b}
Texture	3.50±0.76^{a}	3.38±0.52^{a}	3.63±0.52^{ab}	4.25±0.71^{b}
Colour	5.13±0.35^{b}	5.13±0.99^{b}	3.88±0.99^{a}	5.38±1.19^{b}
Taste	3.88±0.35^{a}	4.88±0.99^{c}	4.25±1.17^{ab}	4.50±0.54^{b}
General Acceptance	3.88±0.99^{a}	4.38±0.74^{b}	4.50±0.93^{b}	4.43±0.99^{b}

* Different letters in the same row indicate significant differences ($p<0.05$); $\overline{X} \pm S_{\overline{X}}$: average ± standard deviation

TABLE 4 - The changes in TVB-N content of precooked crab meat treated with herbs.

Days	Samples			
	Control	Thymus	Rosemary	Laurel
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
1	25.67±0.02^{a}	27.56±0.49^{b}	27.56±0.51^{b}	25.42±0.35^{a}
38	46.12±0.51^{c}	34.36±0.43^{a}	36.21±0.76^{b}	35.31±0.49^{ab}
42	89.21±1.38^{d}	41.24±0.44^{c}	38.05±0.61^{b}	35.41±0.28^{a}
50	58.72±0.49^{a}	60.27±0.50^{b}	58.80±0.50^{a}	61.67±0.49^{c}
57	214.25±7.97^{d}	107.49±5.37^{b}	155.61±13.91^{c}	76.96±0.72^{a}

* Different letters in the same row indicate significant differences ($p<0.05$); $\overline{X} \pm S_{\overline{X}}$: average ± standard deviation

TABLE 5 - The changes in TBA content of precooked crab meat treated with herbs leaves.

Days	Samples			
	Control	Thymus	Rosemary	Laurel
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
1	0.01±0.00^{a}		0.01±0.00^{a}	0.03±0.01^{b}
38	0.03±0.01^{b}	0.01±0.00^{a}	0.02±0.00^{a}	0.04±0.00^{b}
42	0.32±0.03^{c}	0.06±0.00^{a}	0.07±0.01^{a}	0.15±0.00^{b}
50	0.07±0.01^{a}	0.06±0.02^{a}	0.08±0.01^{a}	0.13±0.03^{b}
57	0.07±0.01^{a}	0.08±0.00^{a}	0.08±0.01^{a}	0.14±0.01^{b}

* Different letters in the same row indicate significant differences ($p<0.05$); $\overline{X} \pm S_{\overline{X}}$: average ± standard deviation

TABLE 6 - The changes in pH of precooked crab meat treated with herbs.

Days	Samples			
	Control	Tymus	Rosemary	Laurel
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
1	6.53±0.01^{b}	6.44±0.01^{a}	6.54±0.01^{b}	6.44±0.02^{a}
38	6.27±0.02^{a}	6.27±0.01^{a}	6.40±0.04^{c}	6.34±0.01^{b}
42	6.13±0.02^{a}	6.24±0.01^{b}	6.27±0.01^{c}	6.23±0.01^{b}
50	6.26±0.04^{b}	6.09±0.02^{a}	6.22±0.03^{b}	6.13±0.02^{a}
57	5.86±0.08^{a}	6.03±0.02^{b}	5.98±0.02^{b}	6.01±0.02^{b}

* Different letters in the same row indicate significant differences ($p<0.05$); $\overline{X} \pm S_{\overline{X}}$: average ± standard deviation

TABLE 7 - Biogenic amine contents (mg/100 g) in precooked carb meat.

Groups/ Storage time (days)	Ammonium	Putrescine	Cadaverine	Spermidine	Tryptamine	2-Phenyl-ethylamine	Spermine	Histamine	Seretonine	Tyramine	TMA	Dopamine	Agmatine
Control 1.	58.37±1.97^{a}	1.60±0.14^{a}	3.41±0.16^{ab}	3.00±0.00^{a}	7.13±0.51^{a}	2.34±0.23^{c}	3.00±0.00^{a}	0.00±0.0^{a}	14.10±0.24^{a}	0.00±0.00^{a}	2.86±0.06^{a}	4.24±0.10^{a}	1.41±0.15^{b}
Thymus	55.44±5.23^{a}	2.32±0.11^{ab}	3.48±0.13^{ab}	3.50±0.35^{a}	6.93±0.86^{a}	0.00±0.00^{a}	3.14±0.42^{b}	0.00±0.0^{a}	13.60±0.44^{b}	0.57±0.15^{b}	2.31±0.32^{a}	4.28±0.46^{a}	1.55±0.16^{b}
Rosemary	58.28±3.60^{a}	2.20±0.07^{ab}	4.28±0.53^{b}	3.98±0.29^{a}	6.78±0.92^{a}	1.48±0.30^{b}	4.79±0.33^{c}	0.00±0.0^{a}	14.27±1.22^{a}	0.00±0.00^{a}	2.37±0.02^{a}	6.10±0.34^{b}	1.71±0.19^{b}
Laurel	50.53±4.02^{a}	2.62±0.54^{b}	2.89±0.41^{a}	2.36±1.02^{a}	7.15±0.80^{a}	0.00±0.00^{a}	3.27±0.26^{b}	0.00±0.0^{a}	12.41±0.90^{b}	0.39±0.05^{b}	2.87±0.25^{a}	4.41±0.16^{a}	1.14±0.17^{a}
Control 38.	174.83±12.08^{c}	24.73±1.91^{b}	6.52±0.78^{a}	2.06±0.07^{ab}	3.31±0.29^{a}	0.00±0.00^{a}	4.88±0.24^{b}	0.36±0.09^{b}	5.96±0.64^{a}	11.32±0.79^{c}	7.45±0.70^{b}	4.39±0.23^{a}	2.69±0.25^{a}
Thymus	51.41±5.20^{ab}	2.76±0.36^{a}	5.02±0.39^{a}	1.05±0.01^{a}	3.22±0.47^{a}	0.00±0.00^{a}	4.58±0.26^{b}	0.16±0.03^{a}	7.20±0.57^{ab}	1.65±0.24^{a}	2.75±0.77^{a}	5.48±0.60^{b}	3.44±0.70^{b}
Rosemary	52.02±4.89^{ab}	3.10±0.54^{a}	6.21±0.67^{a}	2.75±0.28^{b}	7.46±0.45^{b}	0.00±0.00^{a}	2.14±0.14^{a}	0.23±0.07^{a}	8.33±0.86^{b}	0.84±0.10^{a}	2.17±0.19^{a}	5.68±0.47^{b}	4.90±0.21^{c}
Laurel	41.90±2.40^{a}	4.23±0.47^{a}	6.56±0.63^{a}	3.63±0.92^{c}	7.02±0.60^{b}	0.00±0.00^{a}	5.32±0.40^{b}	0.17±0.09^{a}	14.67±1.13^{c}	4.54±0.27^{b}	2.53±0.22^{a}	4.94±0.21^{a}	3.10±0.79^{b}
Control 42.	204.55±10.31^{c}	40.33±3.56^{b}	9.46±1.10^{ab}	7.50±2.10^{a}	3.35±0.27^{a}	1.94±0.47^{a}	2.76±0.32^{a}	3.78±0.42^{b}	5.16±0.27^{b}	5.84±0.44^{c}	24.20±1.61^{c}	7.66±0.74^{a}	12.17±0.68^{c}
Thymus	85.65±5.84^{ab}	3.19±0.48^{a}	7.96±0.76^{a}	9.76±0.85^{b}	4.99±0.29^{b}	0.00±0.00^{b}	4.10±0.45^{b}	0.00±0.00^{a}	14.16±0.57^{c}	1.37±0.12^{b}	10.85±1.56^{b}	11.04±1.00^{a}	2.97±0.24^{b}
Rosemary	88.70±6.64^{ab}	4.34±0.17^{a}	9.76±0.58^{ab}	10.22±0.95^{b}	11.57±0.62^{c}	1.22±0.18^{a}	7.29±0.53^{c}	0.00±0.00^{a}	14.85±1.34^{c}	0.29±0.01^{a}	13.60±0.35^{a}	16.57±2.13^{b}	7.58±0.39^{a}
Laurel	72.94±6.13^{a}	5.02±0.71^{a}	10.86±0.67^{b}	6.81±0.71^{a}	6.08±0.45^{b}	0.00±0.00^{b}	7.36±0.22^{c}	0.00±0.00^{a}	10.66±0.10^{a}	3.93±1.15^{c}	10.95±0.96^{b}	14.65±0.77^{b}	7.74±0.19^{a}
Control 50.	238.65±12.14^{c}	61.28±6.06^{b}	12.73±0.96^{a}	6.49±0.35^{a}	4.17±0.42^{a}	0.00±0.00^{a}	2.70±0.40^{c}	7.84±0.66^{c}	6.83±0.78^{a}	0.67±0.17^{a}	41.57±2.65^{c}	2.59±0.23^{a}	0.35±0.04^{a}
Thymus	106.01±4.14^{a}	3.49±0.56^{a}	14.89±1.68^{a}	5.43±1.13^{c}	7.49±0.61^{b}	2.50±0.32^{b}	5.74±0.29^{d}	0.21±0.02^{a}	10.66±0.86^{b}	2.18±0.35^{b}	18.17±2.23^{a}	15.27±0.95^{c}	2.81±0.29^{b}
Rosemary	103.68±5.64^{a}	5.93±0.17^{a}	12.44±1.27^{a}	5.58±0.28^{a}	6.16±0.35^{b}	6.25±0.67^{c}	1.11±0.11^{b}	0.64±0.11^{b}	10.69±0.18^{b}	9.07±0.81^{d}	24.54±1.49^{b}	14.30±0.35^{b}	3.49±0.27^{c}
Laurel	95.24±4.89^{a}	8.65±0.75^{a}	15.41±0.98^{a}	7.67±0.71^{b}	4.14±0.52^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.84±0.06^{b}	7.69±0.77^{a}	7.00±0.62^{c}	20.44±1.63^{a}	14.18±0.41^{b}	2.44±0.27^{b}
Control 57.	311.52±38.54^{c}	88.77±5.38^{b}	18.69±2.37^{a}	10.25±0.64^{a}	9.61±0.63^{b}	0.00±0.00^{a}	4.37±0.40^{b}	10.36±0.21^{c}	1.36±0.17^{a}	12.72±1.11^{b}	100.56±6.78^{c}	9.86±0.98^{b}	2.95±0.14^{b}
Thymus	152.58±7.38^{b}	10.76±1.50^{a}	33.80±2.99^{b}	4.72±0.71^{c}	10.50±1.06^{b}	0.00±0.00^{a}	6.36±0.35^{c}	0.28±0.03^{a}	7.67±0.37^{c}	15.00±1.23^{b}	38.18±0.89^{b}	17.57±1.12^{c}	4.71±0.65^{c}
Rosemary	147.20±5.59^{b}	15.53±1.42^{a}	18.13±1.64^{a}	4.45±0.42^{a}	10.75±0.79^{b}	1.24±0.35^{b}	9.52±0.89^{d}	0.93±0.05^{b}	6.90±0.88^{c}	0.47±0.08^{a}	46.05±0.63^{a}	14.59±0.55^{a}	1.56±0.16^{a}
Laurel	129.32±4.75^{a}	14.70±0.89^{a}	20.51±.93^{a}	7.05±0.42^{b}	2.29±044^{a}	0.00±0.00^{a}	1.90±0.21^{a}	1.30±0.04^{a}	3.43±0.11^{b}	14.71±0.67^{b}	45.02±2.09^{a}	19.59±1.09^{c}	5.73±0.40^{c}

* Different letters (a, b, c, d) in the same column for different storage days indicate significant differences ($p<0.05$).

age period. However, the TVB-N value for the control group increased up to 89.21 mg TVB-N/100 g flesh by day 42 and then started to decrease to 58.72 mg TVB-N/100g by day 50.

Table 5 shows TBA contents in the different treatments during storage. The pH values of samples of precooked crab meat stored at 4 °C are shown in Table 6. With an increase in storage time, pH values decreased. Significant difference was found ($p<0.05$) between the control and the treatment groups in pH of samples during storage period.

3.3. Biogenic amines

The concentrations of the biogenic amines in the muscle of precooked crab meat with or without herbs are given in Table 7.

3.4. Microbiological assessment

Microbial counts of precooked crab meat samples stored at 4 °C are shown in Fig. 1.

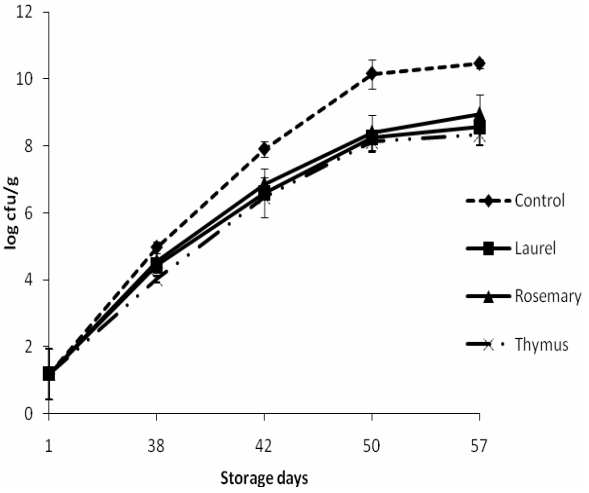

FIGURE 1 - TVC content of precooked crab meat treated with herb leaves.

4. DISCUSSION

4.1. Sensory assessment

As crab products spoil, these typical crab odours and flavours are replaced by volatile compounds, such as ammonia from protein degradation, rancid odour, or off-flavour from lipid oxidation and putrefaction [33].

The application of seasoning with herb leaves to precooked and vacuum-packed crab meat stored at 4 °C led to an improvement in the appearance and odour of the samples, which received higher scores than the control ($p<0.05$) from day 1 onwards (Tables 1-3). Off odour and off flavour caused by microbial activity were detected towards the end of storage period for all groups. According to sensory results, the limit for sensory acceptability of precooked and vacuumed crab meat stored at 4 °C was ~38-40 days for the control whereas the shelf-life of samples with herb leaves was ~ 40-42 days. As spoilage progressed, the off-flavour increased in intensity until the crab meats were no longer edible at 42 days. The rejection point for the samples was below 5 at 42 days, except the sample with laurel leaves ($p<0.05$, general acceptability: 5.13). The use of herb leaves improved the sensory quality of precooked and vacuum-packed crab meats. In addition, similar results for fish species treated with plant extracts were obtained from other studies [34-38].

42. Chemical assessment

Total volatile basic nitrogen (TVB-N) is 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 [39]. The TVB-N values for the treated groups remained low compared to those of the control. The lowest TVB-N value was obtained from the sample with thymus leaves ($p<0.05$) whereas the control group gave the highest TVB-N value ($p<0.05$) at 38 days. There were significant difference ($p<0.05$) in the levels of TVB-N among all the samples at 42 days. The lowest value was obtained from the samples with laurel, followed by rosemary and thymus leaves, at 42 days. The levels of 30-35 mg N per 100 g muscle are considered to be the limit of acceptability for ice-stored cold water fish [40, 41]. Gates *et al.* [42] reported that TVB-N value of fresh blue crab meat was 26.2 mg/100 g, and reached 35 mg/100 g in cooked meat during storage at 0 °C for 18 days. TVB-N and TMA-N levels in the muscle of *post mortem* Chinese mitten crab (*Eriocheir sinensis*) stored at 4 and 20 °C were studied [3], and levels did not change greatly during 72-h storage at refrigeration temperature. Therefore, the authors concluded that TVB-N and TMA-N were not reliable indicators of the freshness of crabs.

As the samples were heat-treated and taken out of vacuum package only at the time of analysis, the meat samples were not exposed to any additional microbial contamination. However, microbial enzymes from the microbial flora were originally present [40], and contributed to a significant increase in TVB-N level during storage period.

Thiobarbituric acid (TBA) is a second breakdown product of lipid oxidation, and widely used as an indicator of degree of lipid oxidation. The concentration of TBA in freshly caught fish is typically between 3 and 5 mg of MDA equivalents per kg flesh, but levels of 5–8 mg of MDA equivalents per kg flesh are generally regarded as the limit of acceptability for fish stored in ice [40, 43]. TBA values indicating rancidity development in the all crab meat remained low (<0.32 mg MDA/kg meat) and below the limit level at which rancid flavours may become evident. This fact can be explained by the low level of fat in crab meat, and also removal of oxygen in the vacuum pack.

Sample pH values in advanced stages of spoilage were lower than that of fresh samples. Similar results for

pasteurised crab cake mix were found, and that pH is not a reliable index of decomposition for pasteurized crab cake mix [44].

4.3. Biogenic amines

The biogenic amine content of fish is useful in estimating the freshness and degree of spoilage of fish and fish products. These amines are found in fresh fish at low levels, and their presence is associated with bacterial spoilage [45, 46]. The biogenic amine content of fish depends on fish species, free amino acid content, the moment of capture and stomach contents at death, since microbial flora varied seasonally [47, 48].

Among the biogenic amines, histamine is potentially hazardous and believed to be the causative agent in Scombroid poisoning [49]. Hungerford [50] reviewed that contamination of fish with histamine is due to mishandling and bacterial production of histamine. Fresh fish contain no histamine; or traces of histamine at levels much lower than 50 mg/kg action levels used in the US [51], or the 100–200 mg/kg action levels used in Europe [52]. In the present study, histamine produced by bacterial decarboxylation of free histidine [45] was not detected until 38 days of storage. After that, histamine level, especially in the control group, reached a hazardous concentration after 50 days of storage. The histamine level remained low in the treated groups throughout storage period ($p<0.05$).

Putrescine levels increased throughout the storage period, with significantly higher increase ($p<0.05$) in the control samples (Table 7). When precooked crab meat was rejected by the sensory panel, the levels of putresine were 24.74 mg/100 g for control group and lower than 5 mg/100 g for the treated groups. Cadaverine levels also increased during storage period. However, there were no significant differences ($p>0.05$) between the control and treated samples until the limit of acceptability (42 days).

However, spermidine, spermine, agmatine and tyramine levels also fluctuated during storage period. The concentrations of 2-phenylethylamine were negligible in the samples. Seratonin, tyramine, and dopamine levels remained lower in control than the other groups during storage period.

Fish contain trimethylamine oxide (TMAO), and the quantity depends on fish species and the environment. TMA is associated with the fishy odour of spoilage, and is part of the spoilage pattern of many fish. TMA is not produced in significant amount at the early stages of chill storage of fish but it appears after 3 or 4 days when the rate of TMA production parallels the bacterial proliferation pattern. Therefore, it is not considered to be suitable for fish stored in ice less than 6 days [53]. Fresh fish has very low TMA (<1.5 mg TMA/ 100 g), but values increase during spoilage. The fish is considered to be stale when the rate of TMA production is higher than 30 mg/100 g cod [54]. In this study, when crab meat was rejected by the sensory panel (~38-40 days), the levels of TMA were 7.45 mg/100 g for the control and <3 mg/10 g for the treated groups. The concentration of TMA increased during storage but it did not exceed the recommended level when the crab meat was still edible.

4.4. Microbiological assessment

Initial total viable counts of cooked crab meat were below 1.5 log cfu g^{-1} (day 0), and population of microorganisms significantly ($p<0.05$) increased to >4 log cfu g^{-1} after 38 days of storage. After 42-days storage, TVC was 7.9 cfu g^{-1} for the control**,** 6.86 cfu g^{-1} for rosemary, 6.60 cfu g^{-1} for laurel, and 6.48 cfu g^{-1} for thymus group. A somewhat higher (10^7 cfu g^{-1}) microbial safety criterion is normally applied for determining storage life of fresh seafood [55]. The shelf-lives of precooked and vacuumed crab meat were approximately ~38-40 days for the control and ~42-44 days for treated groups, indicating that sensory analysis of crab meat correlated well with microbiological analysis.

Precooking (83 ^{o}C for 10 min) reduced the bacterial counts in the products from 4 cfu g^{-1} for raw crab meat to 1.19 cfu g^{-1} for cooked crab meat. Total plate counts of microorganisms in herbs used are 0.9 cfu g^{-1} for thymus, rosemary and laurel leaves. The groups treated with rosemary and laurel leaves showed fairly large counts of bacteria compared to the group with thymus during storage period.

Generally, seasoning with herb leaves improved the sensory quality of precooked and vacuumed crab meat. The results of chemical analyses showed that the application of seasoning also delayed spoilage of crab meat. Sensory analysis correlated well with microbiological analysis, showing a longer shelf-life in the treated samples. This study provides useful information on the use of seasoning in order to improve the sensory quality, and also to delay spoilage of crab meat during storage period.

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Received: October 10, 2011
Accepted: November 14, 2011

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