Chapter 3

Results and Discussion

1. Determination of rigor process and sensory profile (aroma/odor, flavor and texture) of fresh fish and fish stored in ice

1.1 Determination of rigor process

Results in Table 5 show that fish sized 1.1 to 1.2 kg started to rigor within 1-2 hr and had reached full rigor mortis in about 8 hr. A similar trend was found in the large sized fish (data are shown in Table 1, 2; Appendix 2). In addition, the tail part had the highest firmness as compared with the other two parts. This agreed with the results obtained in various parts of farmed rainbow trout fillets (Morkore *et al.*, 2002) and at 8 hr all samples reached the highest firmness. In general terms, it can be concluded that firmness was related to rigor index. However, it cannot be used alone as an indicator without knowing the history of the fish, regarding its size, handling process and location of the samples.

Table 5 Firmness of fish samples (1,100-1,200g) during the rigor process

RI/Rigor stage/ Storage time	Firmness (g force)		
	Position		
	Tail	Middle	Head
RI=0 /Pre-rigor/ 0 hr	206.82 ± 190.06[1] Aa	62.04 ± 5.55 Bb	76.58 ± 13.93 Cb
RI=0.01-0.2 /Rigor (onset) / 1 hr	258.66 ± 141.32 Aa	119.22 ± 12.37 ABb	147.62 ± 32.09 ABab
RI=0.1-0.3/Rigor (onset) / 2 hr	135.26 ± 59.64 Aa	89.68 ± 9.12 Bb	89.58 ± 7.24 Bb
RI=0.5-0.6/Rigor / 5 hr	239.94 ± 203.90 Aa	77.42 ± 9.64 Bc	122.48 ± 27.86 Abb
RI=1/Full rigor / 8 hr	668.84 ± 406.72 Aa	141.46 ± 25.67 Ac	198.20 ± 60.63 Ab

RI = Rigor Index
1 = Mean ± SD
[A] means within the same column not followed by the same letter are significantly different.
[a] means within the same row not followed by the same letter are significantly different.

1.2 Determination of sensory profile (aroma/odor, flavor and texture) of fresh and ice stored fish

Sensory in raw fish

The sensory profile in raw fish is shown in Table 6.

Slime or Mucus

Freshly caught seabass were slimy and translucent with no viscous appearance (Table 6). In life, the slime helps the fish move through water, protects it from minor abrasions (Dore, 1991) defends the fish against pathogens, turbulent water (Rankin and Jensen, 1993) as well as stress (Heath, 1995). The slime on the fish body decreased as storage time increased. This may be due to the effect of melting ice. However, the amount of slime in the gills remained with storage time. However, slime in the gills changed with regards to its viscosity and its opaqueness, which may be a result of bacterial growth.

Gills/ Skin

The gills of freshly caught fish ranged in color from bright red to dark red due to oxyhaemoglobin and haemoglobin form. The gills of ice-stored fish have color ranges from pink to dark brown. The condition of the gills is sometimes useful in judging fish freshness. However, in this experiment, it was found that there was a variety of gills color; bright red, red, dark red, pale red and red with dark spots. Dore (1991) stated that gill color should not be over emphasized as an indicator of fish freshness. The storage time effect on gills color was more important than on the fish body color. This may be due to two main factors: Fish scales may contain less pigment and they also help protect the fish from the oxygen; therefore, melting ice effect and lipoxygenase contained in the skin did not cause the problems. Some researchers found that skin and gills contain lipoxygenase. So they can cause lipid oxidation, which can generate off flavor (German and Kinsell, 1996; Hsieh and Kinsella, 1989; Zhang *et al.,* 1992 a, b). In general, during ice storage, skin not only generates off flavor but also induces discoloration (Mohri *et al.,* 1992). Brightness of body decreased as storage time increased. Odor of gills from freshly caught fish

smelled like fresh algae but this odor changed to fishy then rancid in longer storage time, which may confirm that gills contain enzyme lipoxygenase.

Eyes

The eye was a very good quality indicator of freshly caught fish right up to 2 days of ice storage. Pupils were very bright and fluorescent. No black color could be detected in the fresh fish, as this is typical of seabass. However, during ice storage, it is difficult to determine fish quality by eyes shape such as sunken or swollen. Because the fish side that is in contact with the ice has never been sunken, it is always swollen. On the other hand, the eye on the other side is always sunken, except for the first two days when eye shape is normal.

Scales

The adhesiveness of the scales decreased as storage time increased especially when fish were kept in ice for more than 6 days. This is due to the proteolysis mechanism. So this may provide an alternative indicator for fish quality if there is appropriate mechanical equipment such as texturometer to measure it.

Belly

In general, the belly of uneviscerated fish is checked for swelling and gas production because the contents in the gut may ferment and swell (Dore, 1991). However, it was found that the catching style of seabass might be different from that of ocean fish. Seabass fishermen do not provide much feed to the fish or may stop feeding prior to catching. This may account for the thickness of the belly and skin not bursting, as other fish may tend to do. There was no belly-bursting event in seabass, so the belly should not be used as a freshness indicator.

Table 6 Summary of sensory profile evaluation of raw seabass kept in ice for 14 days

Attribute	Storage time (day)				
	0	1	2	4	6
eyes	convex, transparent cornea bright pupil and fluorescent	convex, transparent cornea bright pupil and fluorescent	convex, slightly opalescent cornea bright pupil	flat slightly opalescent cornea pupil not bright	flat slightly opalescent cornea slightly opaque pupil
gills	bright red to red, transparent slime, mild algae odor to mild fishy	red, transparent slime, mild fishy	red to dark red, slightly sticky slime, mild fishy	pink to dark brown, sticky slime	dark red to dark brown but slightly pale at rim of gills, sticky slime, moderate fishy, mild rancid
body	gray to green gray around the dorsal part, white gray around belly part, bright, transparent slime, firm scale	gray to green gray around the dorsal part, white gray around the belly part, bright, transparent slime, firm scale	green gray around the dorsal part, white gray around the belly part, bright, transparent slime, firm scale	green gray around the dorsal part, white gray around the belly part, slightly dull, slightly cloudy slime	green gray around the dorsal part, white gray around the belly part, dull, slightly cloudy slime, slightly loose scale
texture					
whole body	very firm	very firm	very firm	firm	firm
flesh	very firm translucent shining, metabolic sheen	very firm translucent shining, metabolic sheen	very firm translucent shining	firm translucent shining	less elastic slightly dull surface
belly part (anus)	firm, no bleeding from anus	firm, no bleeding from anus	firm, no bleeding from anus	firm, no bleeding from anus	firm, no bleeding from anus
odor					
gills part	mild algae odor to mild fishy	mild algae odor to mild fishy	mild fishy	mild fishy	fishy
body part	mild algae odor to mild fishy	mild algae odor to mild fishy	mild fishy	mild fishy	fishy

Table 6 Summary of sensory profile evaluation of raw seabass kept in ice for 14 days (continued)

Attribute	Storage time (day)			
	8	10	12	14
eyes	concave in the center for upper eyes but convex for another side, opalescent cornea, opaque pupil	concave in the center for upper eyes but convex for another side, pink spot cornea, opaque pupil	concave in the center for upper eyes but convex for another side, pink and red spot cornea, opaque pupil	concave in the center for upper eyes but convex for another side, red spot cornea, opaque pupil
gills	dark red to dark brown but slightly pale at rim of gills, sticky slime, fishy, rancid	pale, sticky slime, rancid and fishy	pale to dark, sticky slime, strong rancid and fishy	pale, sticky slime, very strong rancid and fishy
body	gray around the dorsal part, white gray around the belly part, dull, cloudy slime, loose scale, pale tail	gray around the dorsal part, white gray around the belly part, dull, cloudy slime, loose scale, pale tail	gray around the dorsal part, white gray around the belly part, dull, cloudy slime, loose scale, very pale tail	gray, dull, cloudy yellow slime, loose, very pale tail
texture				
whole body	firm	firm	firm	slightly soft
flesh	slightly soft, less elastic, left finger print, slightly dull surface	soft, less elastic, left finger print, dull surface	soft, left finger print, dull surface, red spot	soft, left finger print, dull surface, red spot
belly part (anus)	firm, slightly bleeding from anus	firm, slightly bleeding from anus	firm, slightly bleeding from anus	firm, slightly bleeding from anus
odor				
gills part	fishy, slightly rancid	fishy, rancid	strong rancid fishy	very strong rancid fishy
body part	Fishy	fishy	fishy	fishy

Sensory in cooked fish

The sensory attribute in cooked form is shown in Table 7.

Flavor/ Odor

Very fresh fish has a good smell with a slightly sweet odor but flavor decreased as storage time increased. The mild good smell still existed right to the end of the storage time.

Taste

According to the nature of the fish, seabass has a mild, creamy and sweet taste, which lasted for 2 days. But the fish would be tasteless after 9 days of ice storage.

Texture

Consumption size of seabass in Thailand varied from 0.6-3.0 kg. However, market size is about 1.0-1.5 kg. Some consumers and fishermen have mentioned that the bigger the size the firmer the texture. A good quality size is about 2.0-2.5 kg, as anything bigger than this size is tough. Small fish (less than 0.7 kg) have a sloppy texture (personal contact). In this experiment it was found that firmness of fish decreased as storage time increased. This may be one of the reasons for low consumer preference. Softening is due to the proteolytic process, which occurs at post rigor caused by hydrolytic enzymes such as calpains (neutral, cystein proteases) and cathepsins (acid proteases) (Sikorski, 2001). Moreover, juiciness is also affected by the storage time due to loss of water entrapment in the lattice spacing between the myosin and actin filaments (Sikorski, 2001).

Protein coagulate

Protein degradation causes more peptides and amino acids to form a curd-like material on the fish surface and intracellular space after heating. So for steamed fish, one indicator determining fish freshness is protein coagulate similar to curd.

Color of fish

It was found that iced fish meat was pale in color as compared to fresh fish after steaming. This may be due to the loss of moisture/ oil or drip from the fish muscle. Using color of cooked fish as an indicator, a highly experienced panel needs to be used to avoid useless data. As a whole, panels preferred the fish kept in the ratio of ice to fish of 3:1 up to 9 days of shelf-life for whole raw fish, but they still accepted cooked fish kept in ice for 12 days.

Table 7 Summary of sensory attributes of cooked seabass kept in ice for 14 days

Attribute	Storage time (day)								
	0	1	2	4	6	8	10	12	14
meat color	light yellow no curd	light yellow no curd	milky no curd	milky no curd	pale white no curd	pale white no curd	pale white curd on meat surface	very pale white curd on meat surface	very pale white curd on meat surface
volume of cooked drip	little-much	little-much	little-much	little-moderate	little-moderate	moderate	little	little	little
appearance of cooked drip	transparent, oil drop	transparent , oil drop	transparent , oil drop	transparent , oil drop	transparent, oil drop	transparent	slightly cloudy	transparent	transparent
flavor sweet	moderate sweet	moderate sweet	moderate sweet	moderate sweet	moderate sweet	moderate sweet	slightly sweet	very slightly sweet	very slightly sweet
fishy	no-very little	no-very little	no-very little	no-very little	no-very little	slightly fishy	fishy	moderate fishy	strong fishy
putrid	no	no	no	no	no	no	no	no	no
off-flavor	no-lightly muddy	no	no	no	no	no	no	no	no
acid flavor	no	no	no	no	no	no	no	no	no
sulfite	no	no	no	no	no	no	no	no	no
taste cream- sweet	cream-sweet	cream-sweet	cream-sweet	cream-sweet	mild cream-sweet	mild cream -sweet	slightly cream-sweet	slightly sweet	plain or tasteless
brittle	no	no	no	no	no	no	no	no	no
sour	no	no	no	no	no	no	no	no	no
texture softness	very firm, juicy	firm, juicy	firm, juicy	firm, juicy	soft, less juicy	soft, less juicy	very soft	very soft	very soft
chewiness	resistant to chewing and produced a typical sound	resistant to chewing	resistant to chewing	soft, less resistant to chewing	soft, less resistant to chewing	soft, less resistant to chewing	very soft, dry	very soft, dry	very soft, dry

Note 1 : Panelists prefer cooked fish stored for 0-1 day because of its firm texture, cream-sweet and good flavor.

2 : Meat color of cooked fish stored for 0-10 days is similar

3 : Texture of cooked fish stored for 8 days is very soft.

4 : All panelists accepted cooked fish stored for 12 days and 80% of them still accepted cooked fish stored for 14 days.

2. Effects of the killing methods, icing delay and parts of the flesh fish on its shelf-life quality

2.1 Killing by immediate immersion in ice-water slurry

Firmness

Fig. 4 shows that the tail part had the highest firmness as compared with other parts, which agreed with the previous result (section 1.1). Firmness of raw fish muscle has a direct relationship to the content of collagen (Sato *et al.*, 1986; Hatae *et al.*, 1986). Furthermore, Morkore *et al.* (2002) explained that the firmness might be due to the difference in collagen content, collagen types and fiber diameter. The major fibrillar collagen of bony fish skin, collagen type, is found to have a subunit composition of α1(I), α2(I) and α3(I); α3(I) known to be a unique subunit of bony fish type I collagen (Sato *et al.*, 1988), while type V collagen is found in trout muscle (Sato *et al.*, 1991). Ando *et al.* (1999) found that the average diameter of collagen fibril for pelagic fish (20.6 $\pm$ 3.0) was smaller ($p<0.05$) than that of demersal fish (26.3 $\pm$6.3) and the differences in collagen fibril diameter might have a relationship with collagen fibril stability. In addition, there are possible explanatory mechanisms of post-mortem softening: some major components within either the myofibrils or in the intracellular connective tissue degrade; or links, bonds and connections that organize and stabilize the structure between the muscle components degrade such that it weakens pericellular connective tissue, which consists of collagen fibril (Ando *et al.*, 1992); or both of these mechanisms occur (Bremner, 1992). Post-mortem disintegration of collagen fibril has been reported to be due to the degradation of the type V collagen molecule (Sato *et al.*, 1997). However, the major component in the muscle of all fish examined so far is type I collagen while type V is a minor component (Sivakumar *et al.*, 2000; Nishimoto *et al.*, 2003). The function of both (type I and type V) collagens is to aid swimming movement and flexibility and changes in their ratios have also been associated with post-mortem changes (Sivakumar *et al.*, 2000). Moreover, cross-linking and stability of collagen increased in response to a stressful environment (Sivakumar *et al.*, 2000). Bleeding is another factor that caused the delay of muscle softening in yellow tail, horse mackeral, and striped jack, which are pelagic fish. Conversely, bleeding had no influence on the

muscle firmness in terms of breaking strength, maximum force of red sea bream, flatfish, and redder fish (Ando *et al.,* 1999) In addition, Jabarsyah *et al.* (1999) found that pink muscle gave higher contractile percentage than white muscle. From observation, it was found that the tail part was high in both collagen and dark pigment. However, the firmness of the tail part did not decrease significantly with storage time, while the middle and head parts had less firmness as storage time increased. This agreed with linear regression analysis (R^2). R^2 of tail, middle and head parts were 0.0149, 0.4532 and 0.6539 respectively. The reason why the tail did not show storage time effect may be due to high collagen content causing higher persistence hydrolysis than other fish parts containing high myofibrillar muscle such as the head or middle parts. The results also showed that a chilling delay for 45 min did not have clear effects on the firmness property of fish. Therefore it should be quite safe for the industry should that occur.

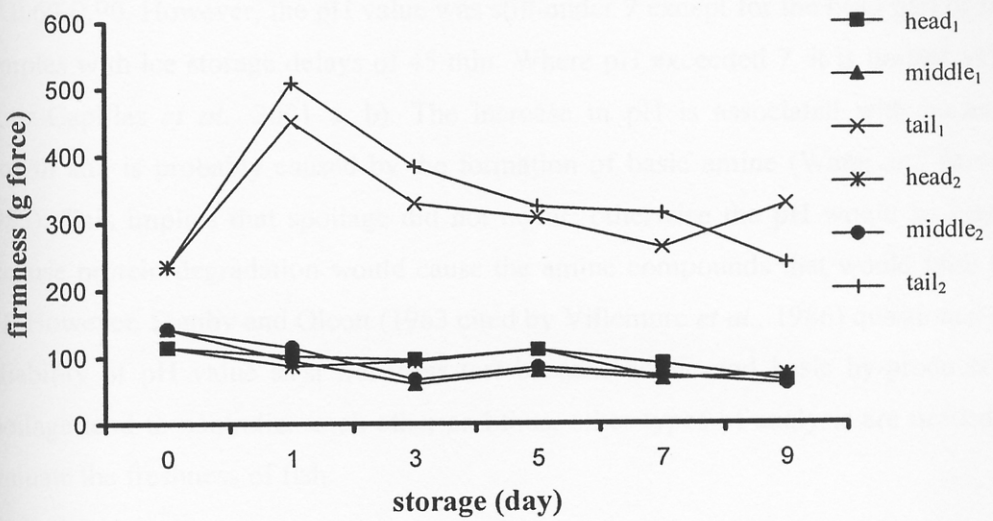

Fig. 4 Firmness of fish killed by ice water and kept in ice for 9 days

1: fish sample without ice storage delay

2: fish sample with ice storage delay for 45 min

pH changes

pH change was shown in Fig. 5. There was no difference in pH found in both treatments ($p>0.05$). Immediately after death, fish had pH around 6.47-6.61 depending on the position of the flesh. In general, the head and middle parts tended to have higher pH than the tail part in the first few days. Morkore *et al.* (2002) found that muscle pH of raw farmed rainbow trout fillet was lower in the neck than in the tail and belly flap but this pattern was not significant in frozen fillets. Different pH found in different fillet parts may, therefore, suggest that glycogen content is unevenly distributed in the fish fillet and/ or breakdown rate of glycogen might differ among the fillet section. Some researchers found that glycogen content, which caused lactic acid accumulation, was high in the tail part compared to the head part (Johnston *et al.*, 1977 cited by Jabarsyah *et al.*, 1999). The pH was lowest on day 1 when kept in ice storage. This may be due to lactic acid accumulation and also ATP degradation (Jabarsyah *et al.*, 1999). After day 1, pH in each part increased with storage time with R^2 0.60-0.90. However, the pH value was still under 7 except for the head part of fish samples with ice storage delays of 45 min. Where pH exceeded 7, it is limited value (Ruiz-Capillas *et al.*, 2001 a, b). The increase in pH is associated with bacterial growth and is probably caused by the formation of basic amine (Wang and Brown, 1983). This implies that spoilage did not occur; otherwise the pH would be higher because protein degradation would cause the amine compounds that would raise the pH. However, Stanby and Olcott (1963 cited by Villemure *et al.*, 1986) questioned the reliability of pH value as a freshness test because acidic and basic by-products of spoilage tend to neutralize each other and thus, other types of analysis are needed to evaluate the freshness of fish.

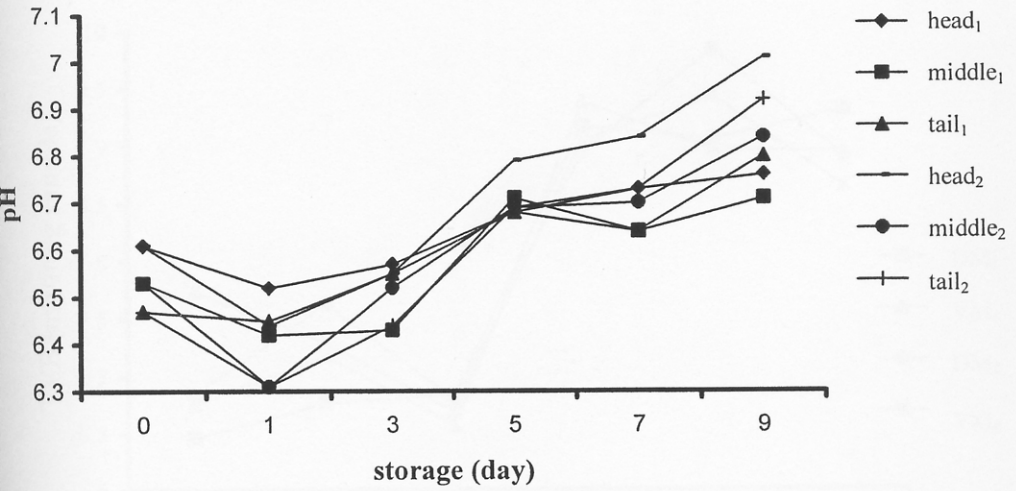

Fig. 5 Changes in pH in different parts of fish killed by ice water and kept in ice for 9 days

1 : fish sample without ice storage delay

2 : fish sample with ice storage delay for 45 min

TVB changes

There was no significant difference in TVB, which comprised the volatile amines (mainly Dimethylamine, Trimethylamine and ammonia) in both treatments as shown in Fig. 6. Results were the same in ventral and dorsal parts. However, ice storage time was one of the main factors affecting TVB content. TVB increase was significantly different ($p<0.05$) after keeping fish in ice for 5 days, but its content was still under the standard value at 25 mg /100 g sample for general fish (Ben-gigirey *et al.*, 1999). EEC (95/145 cited by Ruiz-Capillas *et al.*, 2002) sets a limit for the TVB value for fish at 40 mg /100 g in muscle. There was no legal TVB limit for cephalopod. Considering the TVB content, it can be concluded that fish retains a good quality for consumption for more than 9 days. This agrees with previous studies. The difference in TVB content could also be due to the analytical method used (Villemure *et al.*, 1986).

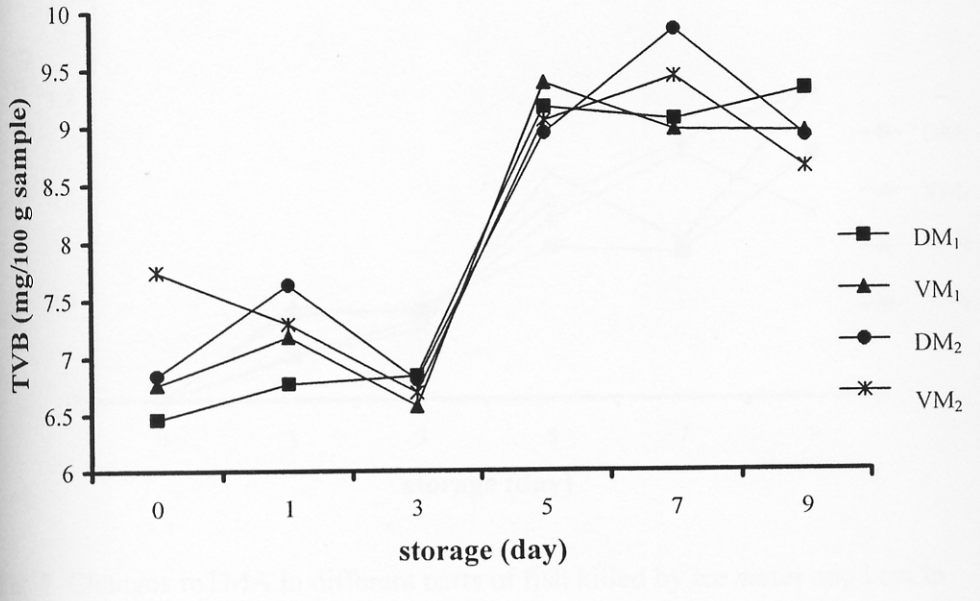

Fig. 6 Changes in TVB in different parts of fish killed by ice water and kept in ice for 9 days

DM_1: dorsal meat of fish sample without ice storage delay

VM_1: ventral meat of fish sample without ice storage delay

DM_2: dorsal meat of fish sample with ice storage delay for 45 min

VM_2: ventral meat of fish sample with ice storage delay for 45 min

TMA changes

TMA in seabass kept in ice storage was very low, just about 1.3 mg /100 g sample as shown in Fig. 7. It can be interpreted that seabass is not a source of TMAO because it is not seawater fish or that there was none or low microbiological degradation of TMAO to TMA, which is consistent with the low bacterial load as described in Table 6. The legal limit of TMA is 12 mg /100 g muscle (EEC directive 91/493 cited by Ruiz-Capillas *et al.*, 2002). However, after 5 days TMA increased significantly, which agrees with the increase in TVB. Moreover, it was also found that TVB was significantly correlated to TMA ($p < 0.05$).

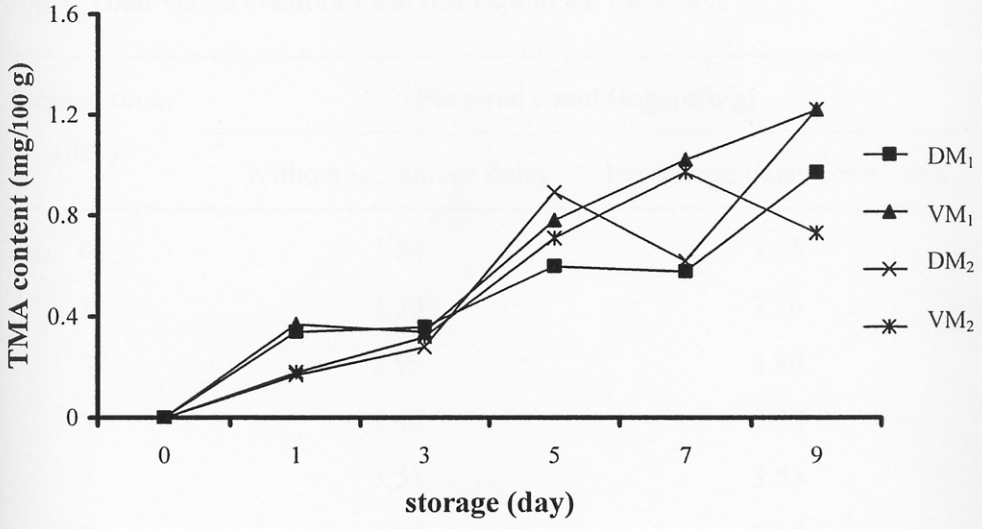

Fig. 7 Changes inTMA in different parts of fish killed by ice water and kept in ice for 9 days

DM_1 : dorsal meat of fish sample without ice storage delay

VM_1 : ventral meat of fish sample without ice storage delay

DM_2 : dorsal meat of fish sample with ice storage delay for 45 min

VM_2 : ventral meat of fish sample with ice storage delay for 45 min

Microbiological changes

The initial load of fresh seabass was about 10^3 cfu/g. It remained at this level throughout the experiment as shown in Table 8. This very low level may be due to the hygienic use of ice and melting ice to preserve the fish. In addition, sea bass is a tropical fish, whereby mesophilic bacteria may be strongly affected by the low temperature in ice storage. So for microbiological quality, it was necessary to determine changes in the psychrophilic bacterial counts in parallel with mesophilic bacterial counts during ice storage in the next experiment.

Table 8 Total viable count of fresh fish kept in ice for 9 days

Storage time (day)	Bacterial count (log_{10} cfu/g)	
	Without ice storage delay	Ice storage delay for 45 min
0	3.54	3.15
1	3.30	3.26
3	2.99	3.80
5	3.46	3.28
7	3.51	3.53
9	3.68	3.95

2.2 Killing by keeping in black bag (anoxia condition)

Firmness

Firmness of fish fillet was shown in Fig. 8 (a, b). There was no difference between the two treatments based on the delay of ice storage ($p>0.05$). Regardless of the position of piece of fillet, the firmness had high fluctuations during storage time. This disagreed with the result of section 2.1. This may be due to higher acid accumulation as compared with the experiment in section 2.1 (fish were killed by ice water) where fish were softer. In general, the firmness of any fish part was lower the initial time (1 hr after keeping in black bag) compared to a previous study. It may be due to the fact that rigor mortis did not occur except for the tail part. Or, acidicity in the muscle may cause weakening of collagen. Furthermore, firmness tended to increase during the first 5 days which differs from previous studies in which firmness tended to decrease as storage time increased. A possibility for this different result may be the difference in killing method. Head ventral and tail had higher firmness than other parts ($p<0.05$), which may be due to the high collagen content that provided higher persistent hydrolysis than the parts containing less muscle collagen such as the head or middle part. However, this variation among the fish parts is one of the factors that should be of concern. Chilling delay for 45 min did not clearly affect this firmness property, which is beneficial to the industry, should this delay occur.

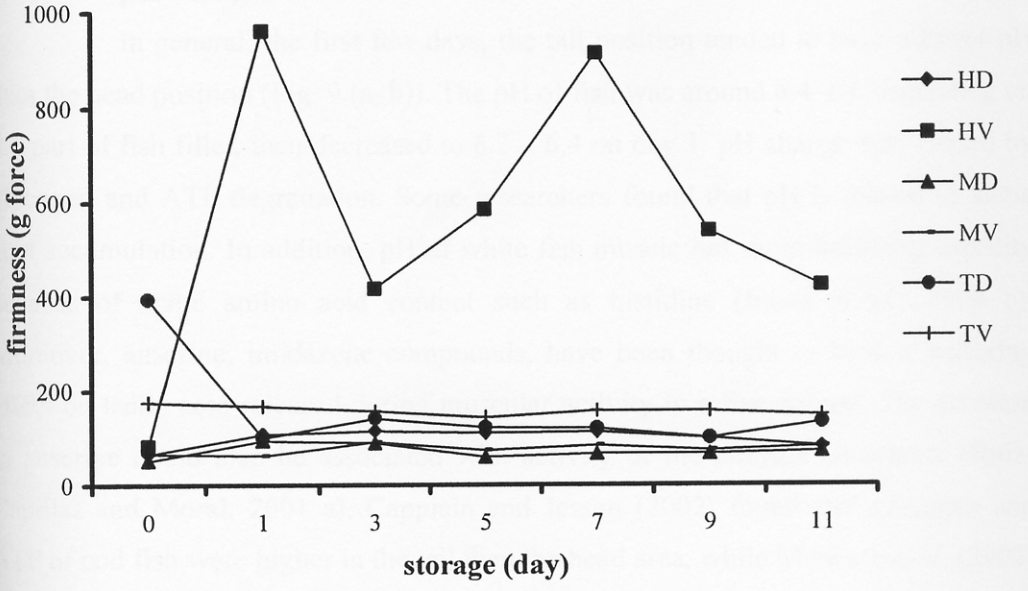

Fig. 8(a) Firmness of different parts of fish killed by anoxia and kept in ice (without ice storage delay) for 11 days

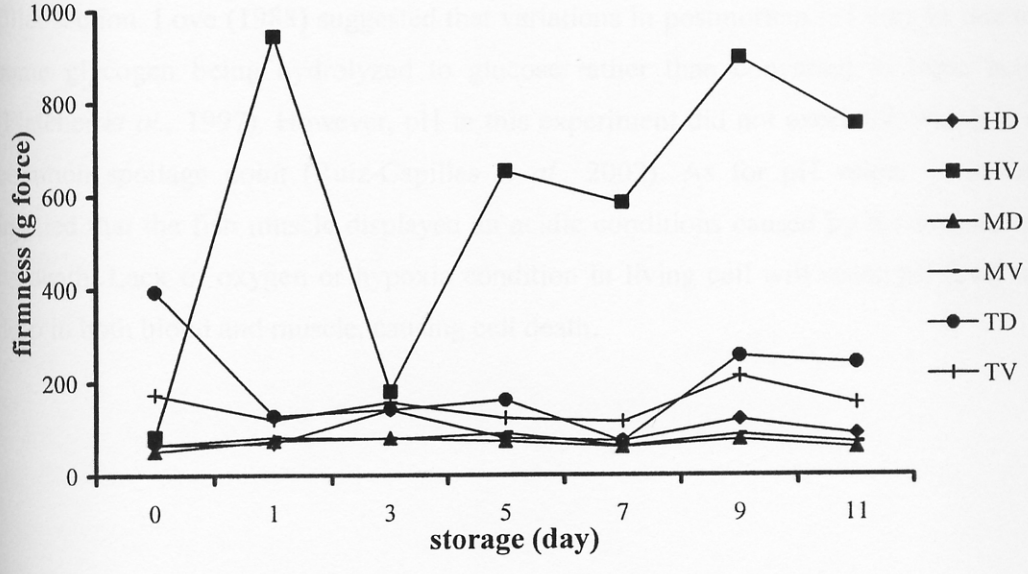

Fig. 8(b) Firmness of different parts of fish killed by anoxia and kept in ice (ice storage delay for 45 min) for 11 days

pH changes

In general, the first few days, the tail position tended to have a lower pH than the head position (Fig. 9 (a, b)). The pH of fish was around 6.4–6.6 depending on the part of fish fillet, then decreased to 6.2 – 6.4 on day 1. pH change was caused by glycogen and ATP degradation. Some researchers found that pH is related to lactic acid accumulation. In addition, pH of white fish muscle has more buffering capacity because of some amino acid content such as histidine (Inoue *et al.,* 1998 b). Moreover, anserine, imidazone compounds, have been thought to have a buffering effect on lactic acid released during muscular activity in a live animal. The decrease in anserine could also be associated with activity of the enzyme anserinase (Ruiz-Capillas and Moral, 2001 a). Capplein and Jessen (2002) found that glycogen and ATP of cod fish were higher in the tail than the head area, while Morkore *et al.* (2002) reported that muscle pH of raw rainbow trout fillets was lower in the neck than in the tail part and belly flap, but his pattern was only significant in unfrozen fillets. Reduction of pH is caused by the formation of lactate from the breakdown of muscle glycogen (Love, 1988 cited by Morkore *et al.,* 2002). Different pH found in different fillet parts may therefore suggest that glycogen content is unevenly distributed in the fillet of living rainbow trout, and /or that breakdown rate post-mortem differs among fillet section. Love (1988) suggested that variations in postmortem pH may be due to some glycogen being hydrolyzed to glucose rather than converted to lactic acid (Fletcher *et al.,* 1997). However, pH in this experiment did not exceed 7, which is a common spoilage point (Ruiz-Capillas *et al.,* 2002). As for pH value, it can be implied that the fish muscle displayed an acidic conditions caused by the manner of its death. Lack of oxygen or hypoxia condition in living cell will cause pH level to drop in both blood and muscle, causing cell death.

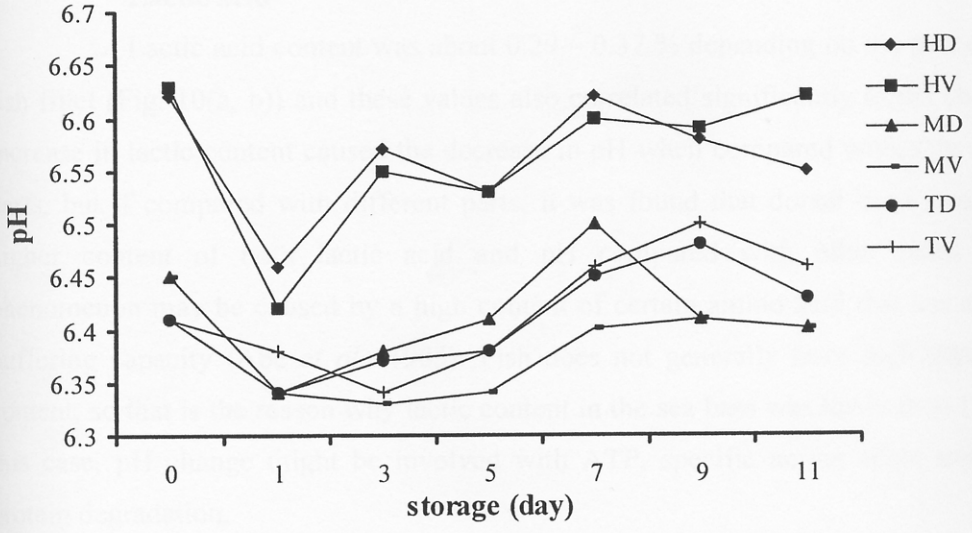

Fig. 9(a) pH changes in different parts of fish killed by anoxia and kept in ice (without ice storage delay) for 11days

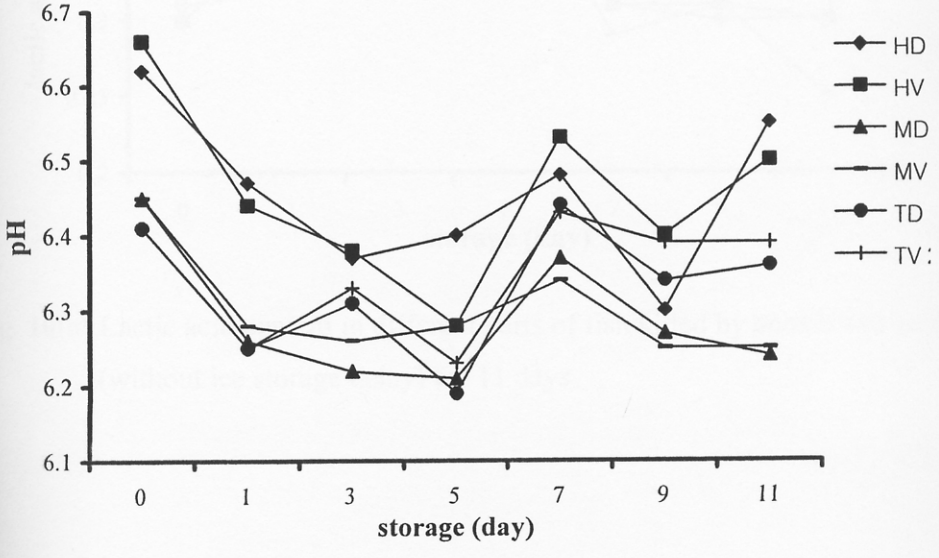

Fig. 9(b) pH changes in different parts of fish killed by anoxia and kept in ice (with ice storage delay for 45 min) for 11 days

Lactic acid

Lactic acid content was about 0.29 – 0.37 % depending on the part of the fish fillet (Fig. 10(a, b)) and these values also correlated significantly to pH change. Increase in lactic content caused the decrease in pH when compared within the same parts, but if compared with different parts, it was found that dorsal head meat had higher content of both lactic acid and pH compared with other parts. This phenomenon may be caused by a high content of certain amino acid that has a high buffering capacity (Abe *et al.,* 1985). Fish does not generally have high glycogen content, so that is the reason why lactic content in the sea bass was lower than 1%. In this case, pH change might be involved with ATP, specific amino acids and also protein degradation.

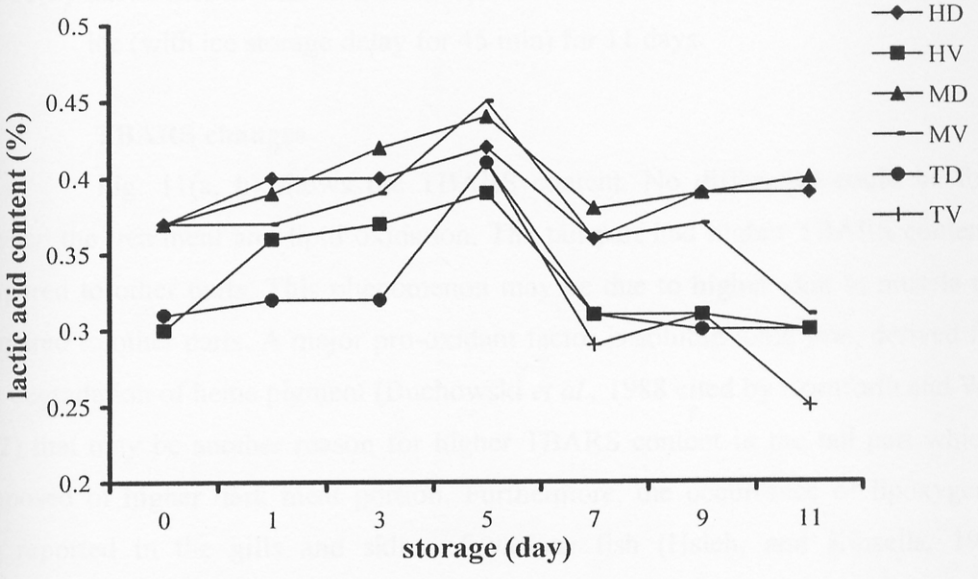

Fig. 10(a) Lactic acid content in different parts of fish killed by anoxia and kept in ice (without ice storage delay) for 11 days

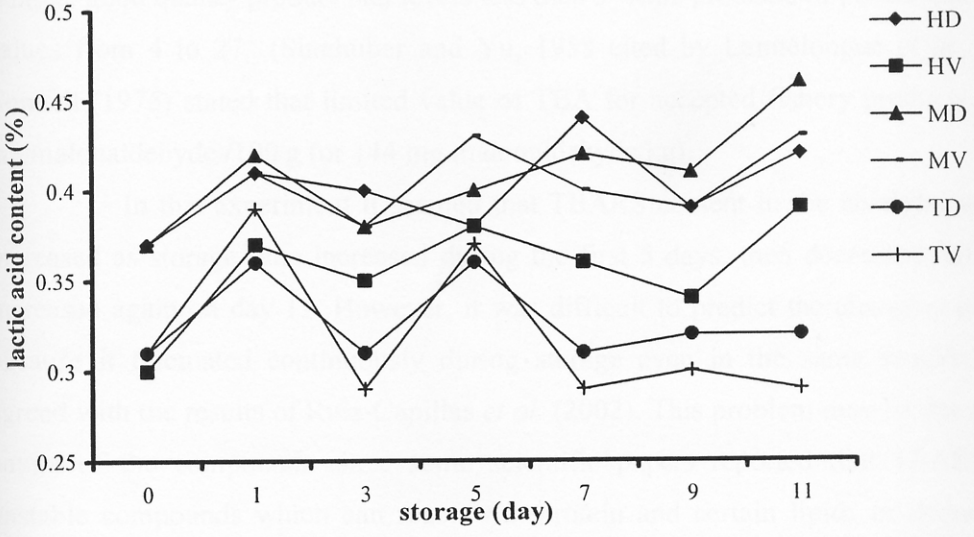

Fig. 10(b) Lactic acid content in different parts of fish killed by anoxia and kept in ice (with ice storage delay for 45 min) for 11 days

TBARS changes

Fig. 11(a, b) shows the TBARS content. No difference could be found between the treatment and lipid oxidation. The tail part had higher TBARS content as compared to other parts. This phenomenon may be due to higher skin to muscle ratio compared to other parts. A major pro-oxidant factor is soluble ionic iron, derived from heat degradation of heme pigment (Buchowski *et al.*, 1988 cited by Cornforth and West, 2002) that may be another reason for higher TBARS content in the tail part which is composed of higher dark meat portion. Furthermore, the occurrence of lipoxygenase was reported in the gills and skins of various fish (Hsieh, and Kinsella, 1989). Moreover, it was found that skin in this experiment had a higher fat content (2-4%) than fish muscle (1-2 %), coinciding with the results of Hultin (1988) who found that enzymatic lipid oxidation in dark muscle of herring was more than 3-4 times that of light muscle. Malonaldehyde levels or thiobarbituric acid (TBA) number, which correspond to acceptable levels of rancidity, are not fully agreed upon since they seem to vary in both the food being analyzed and the method of analysis used (Lannelongue *et al.*, 1982). This agreed with Ruiz-Capillas and Moral (2001 b, c) who found that malonaldehyde did not relate well to rancidity in certain food kept in a controlled atmosphere. However, TBA number expressed as mg of malonaldehyde per 100 g of

sample, good quality product had levels less than 3 while products of poorer quality had values from 4 to 27 (Sinnhuber and Yu, 1958 cited by Lannelongue *et al.,* 1982). Connell (1975) stated that limited value of TBA for accepted fishery products is 14.4 mg malonaldehyde /100 g (or 144 mg malonaldehyde/kg).

In this experiment it seemed that TBARS content in the head dorsal part increased as storage time increased during the first 5 days, then decreased before it increased again on day 11. However, it was difficult to predict the changing pattern because it fluctuated continuously during storage even in the same sample. This agreed with the results of Ruiz-Capillas *et al.* (2002). This problem may be due to the nature of the compounds since some scientific papers reported that TBARS are unstable compounds which can react with protein and certain lipids or degrade to other compounds, producing covalent links and cross-linking of large molecule (Labuza, 1971 cited by Ruiz-Capillas *et al.,* 2002; Aubourg, 1993 cited by Tironi *et al.,* 2002). In general, high temperature and low pH during the reaction may cause an artificial formation of lipid peroxidation products, in which case some antioxidants such as tocopherols and synthetic BHT can be used to reduce that problem (Sheu *et al.,* 2003). However, the use of different BHT concentrations (0 to 1%) in the human hair samples had no clear change in the absorbance of TBARS. Moreover, it was found that some substances would interfere with the determination by reacting with TBA at certain concentrations (Sheu *et al.,* 2003). Accurate TBARS values can be obtained using only a few methods such as Yamaguchi's method because fish contain a large amount of polyunsaturated fatty acids, which are very labile and are easily oxidizable in their tissue (Sakai *et al.,* 1999). It was found that the high performance liquid chromatography is the best method determination of malonaldehyde content because it is sensitive, specific and simple. From our observations, it was found that fish skin was one of many factors affecting the variation of TBARS content in the samples because it was difficult to homogenize the samples. Other reasons may be due to the fact that more O_2 was added in the sample during blending with acid solution. So, further investigation is needed to eliminate factors interfering with TBARS content determination.

Malonaldehyde levels or thiobarbituric acid (TBA) numbers corresponding to acceptable levels of rancidity are not fully agreed upon since they seem to vary with both the food being analyzed and the method of analysis used (Lannelongue *et al.,* 1982). However, in using TBA number, expressed as mg of malonaldehyde per 100 g of sample, good quality product had levels less than 3 while products of poorer quality had values from 4 to 27 (Sinnhuber and Yu, 1958 cited by Lannelongue *et al.,* 1982). So, in this experiment results implied that variability of TBARS was high and therefore, was not a suitable index for lipid oxidation.

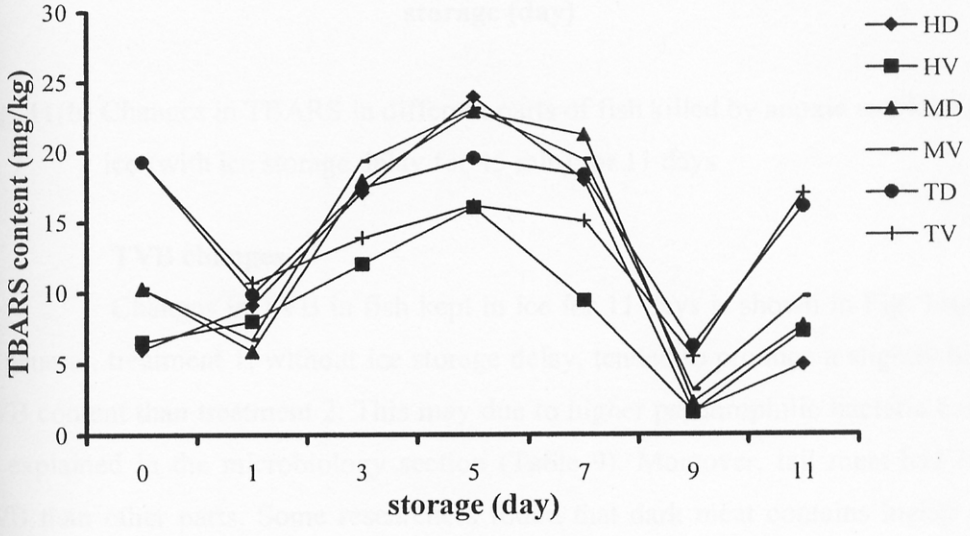

Fig. 11(a) Changes in TBARS in different parts of fish killed by anoxia and kept in ice (without ice storage delay) for 11 days

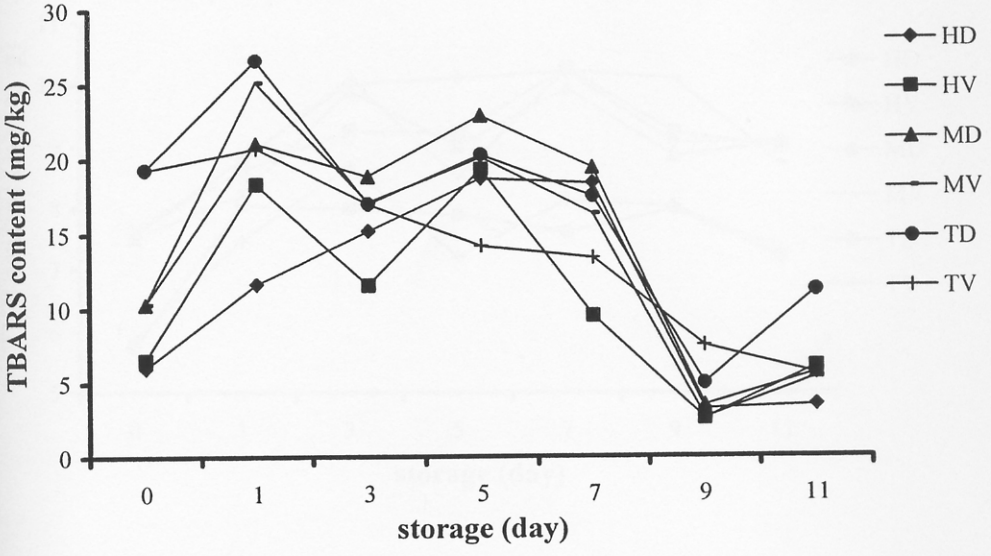

Fig. 11(b) Changes in TBARS in different parts of fish killed by anoxia and kept in ice (with ice storage delay for 45 min) for 11 days

TVB changes

Changes in TVB in fish kept in ice for 11 days is shown in Fig. 13(a, b). In general, treatment 1, without ice storage delay, tended to produce a slightly higher TVB content than treatment 2. This may due to higher psychrophilic bacteria content as explained in the microbiology section (Table 9). Moreover, tail meat had lower TVB than other parts. Some researchers found that dark meat contains higher non-protein nitrogen than light meat (Mai and Kinsella, 1997; Hoffman *et al.*, 1994). However, in this experiment it was found that the tail part had a lot of collagen, so it may have caused the low non-protein nitrogen in total weight form without collagen subtraction. Unless, the collagen was separated from the muscle, the volatile-based nitrogen would definitely occur. TVB tended to increase with storage time, but variation in individual fish should also be considered.

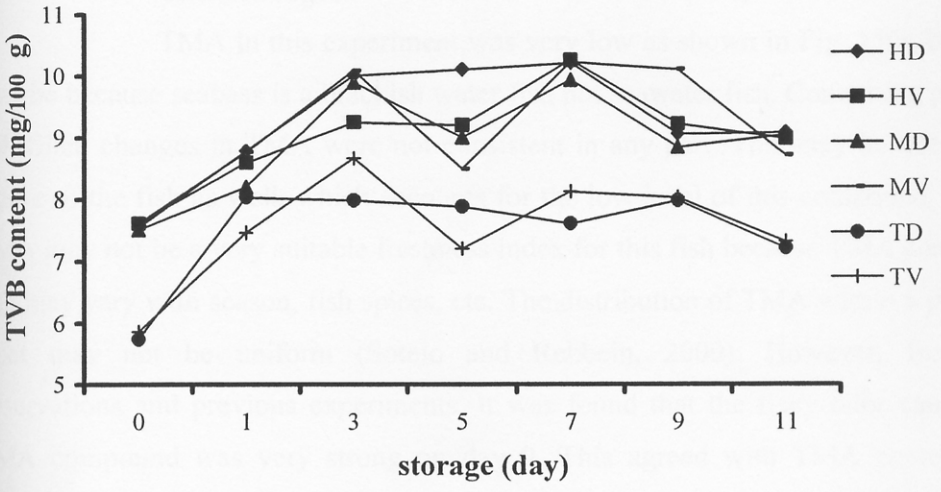

Fig. 12(a) Changes in TVB in different parts of fish killed by anoxia and kept in ice (without ice storage delay) for 11 days

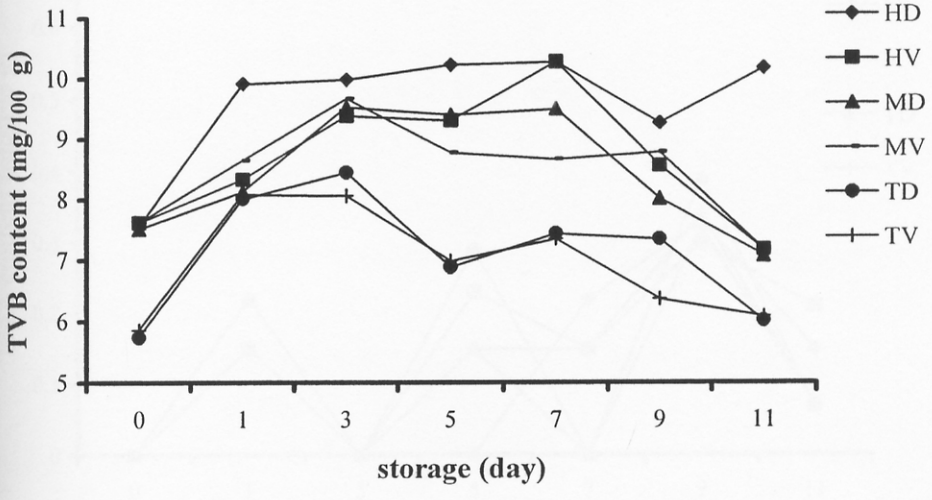

Fig. 12(b) Changes in TVB in different parts of fish killed by anoxia and kept in ice (with ice storage delay for 45 min) for 11 days

TMA changes

TMA in this experiment was very low as shown in Fig. 13(a, b). This may be because seabass is a brackish water fish not seawater fish. Comparing parts of fish fillet, changes in TMA were not consistent in any part. This may be due to the nature of the fish as well, which accounts for the low level of this compound. So the TMA may not be a very suitable freshness index for this fish because TMA content in fish may vary with season, fish spices, etc. The distribution of TMA within a piece of fillet may not be uniform (Sotelo and Rehbein, 2000). However, from the observations and previous experiments, it was found that the fishy odor caused by TMA compound was very strong on day 9. This agreed with TMA content that appeared in varying pieces of fish fillet even though its value was still low. Farber (1965 cited by Lannelongue *et al.,* 1982) found that sole spoiled when the TMA concentration exceeded 4.7 mg of TMA-N/100 g of tissue. Reduction of TMAO is a common property of gram-negative psychrotropic organism (Vandezant *et al.,* 1970 cited by Lannelongue *et al.,* 1982).

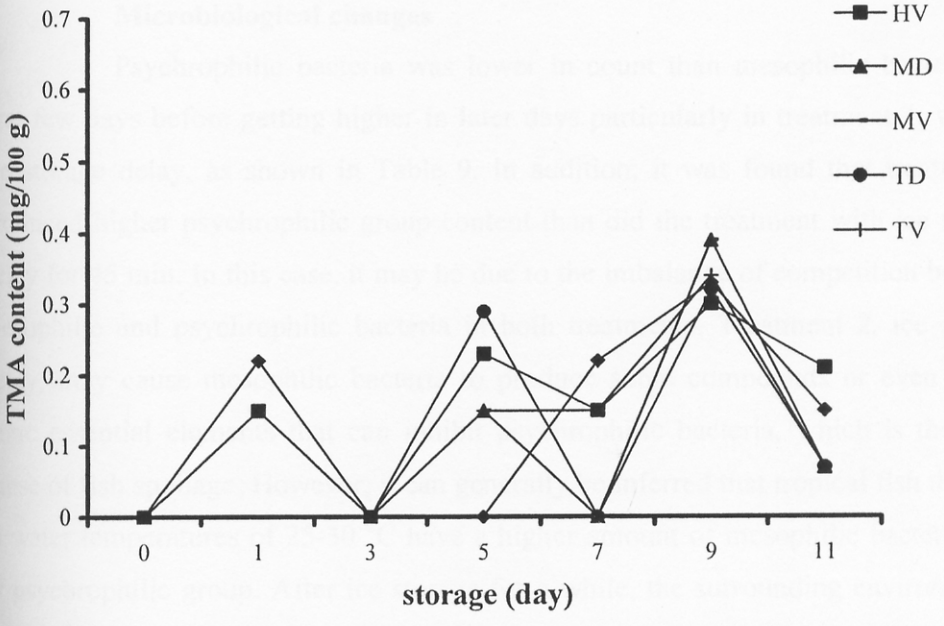

Fig. 13(a) Changes in TMA in different parts of fish killed by anoxia and kept in ice (without ice storage delay) for 11 days

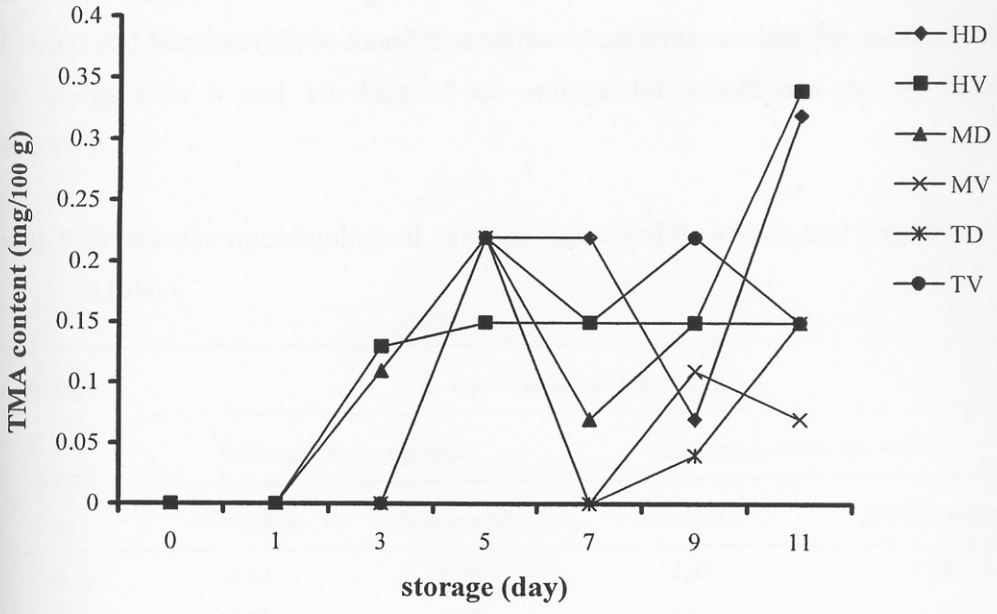

Fig. 13(b) Changes in TMA in different parts of fish killed by anoxia and kept in ice (with ice storage delay for 45 min) for 11 days

Microbiological changes

Psychrophilic bacteria was lower in count than mesophilic bacteria the first few days before getting higher in later days particularly in treatment 1, without ice storage delay, as shown in Table 9. In addition, it was found that treatment 1 produced higher psychrophilic group content than did the treatment with ice storage delay for 45 min. In this case, it may be due to the imbalance of competition between mesophilic and psychrophilic bacteria in both treatments. Treatment 2, ice storage delay, may cause mesophilic bacteria to produce some compounds or even to use some essential elements that can inhibit psychrophilic bacteria, which is the main cause of fish spoilage. However, it can generally be inferred that tropical fish that live in water temperatures of 25-30 ^{o}C have a higher amount of mesophilic bacteria than of psychrophilic group. After ice storage for a while, the surrounding environmental changes would allow psychrophilic bacteria to grow better while this condition causes the inhibition of mesophilic bacteria. Therefore in ice-stored fish, psychrophilic bacterial count should be a good index to determine fish spoilage, while mesophilic bacteria count should be used for food safety in terms of human pathogens because

the optimal temperature for its growth is similar to that of human body temperature. Al-Dagal and Bazaraa (1999) found that untreated controls reached the spoilage onset (10^7 cfu/g) after 6 and 10 days of ice storage for whole and peeled shrimp, respectively.

Table 9 Changes in microbiological value of fish killed by anoxia and kept in ice for 11 days

Storage time (day)	Bacterial count (log_{10} cfu/g)			
	Without ice storage delay		Ice storage delay for 45 min	
	Mesophile	Psychrophile	Mesophile	Psychrophile
0	4.83	3.06	4.83	3.06
1	4.57	2.50	4.73	2.38
3	3.91	3.47	4.84	3.31
5	3.32	3.14	4.08	3.22
7	2.70	3.74	3.31	3.29
9	3.08	4.32	3.07	3.78
11	4.91	6.31	3.71	4.87

3. Natural preservative property of Tom-Yum mix and its ingredients

3.1 Antibacterial and antioxidant properties of β-carotene and isoflavone in the spices of Tom-Yum mix

Bacterial load of different samples are presented in Table 10. The results show that lemon grass and galangal root were heavily contaminated with bacteria, while garlic and shallot had low bacterial loads. Spices and natural seasonings of agricultural material are commonly contaminated with microorganisms including bacteria, mold and yeasts (Alemela *et al.*, 2002). However, the number and type of microorganisms vary with material, origin, climatic conditions, harvesting, storage transport methods used, packaging, and general environmental and handling circumstances, including the nature and extension of quality control measures. Although all spices may be contaminated with microorganisms, when they were used

for formulation as Tom-Yum mix, the formula exhibited reduction in bacterial count correlating with the garlic combinations, which will be described later. Because the results obtained from PCA and TSA are the same in terms of amount of viable count and dimension of clear zone, then PCA was used instead of TSA, or both were used. In addition, bacterial load in the range of 10^5-10^7 cfu /ml is suitable for the determination of the disk diffusion test.

Table 10 Bacterial load in various samples

Sample	Bacterial count [1]
Garlic	1.30×10^1 - 9.20×10^1 cfu/g
Shallot	1.50×10^1 - 9.30×10^1 cfu/g
Kaffir lime leaves	2.31×10^4 - 6.73×10^5 cfu/g
Red chili	5.72×10^3 - 4.81×10^4 cfu/g
Green chili	6.32×10^3 - 7.21×10^4 cfu/g
Galangal (young part)	2.64×10^4 - 7.71×10^6 cfu/g
Galangal (old part)	1.31×10^2 - 4.75×10^4 cfu/g
Lemon grass	4.87×10^4 - 6.97×10^7 cfu/g
Fresh Tom-Yum mix	1.25×10^2 - 3.68×10^4 cfu/g
Fresh Tom-Yum mix (juice)	1.20×10^1 - 6.10×10^2 cfu/ml
Commercial Tom-Yum product	nd
3.5% citric acid	nd
4.5% citric acid	nd
5.5% citric acid	nd

[1]Triplicate plates were tested for each sample in each experiment and each sample was run from three different lots.
nd: not detected

Antibacterial activities

Antibacterial activities of test samples are presented in Table 11. The results indicated that garlic has the strongest inhibitive effect among Tom-Yum ingredients. Some researchers believe that allicin (diallyl thiosulfinate) provides the antimicrobial compound of fresh crushed garlic (Ankri and Mirelman, 1999; Miron *et al.,* 2000). However, when the garlic had been heated there was no inhibition zone around the test bacteria. It is possible that this compound is not heat stable or it may convert to some other form that has no or little antimicrobial activity, which agree with the results of Wilkinson (1997); Ankri and Mirelman (1999) and Prasad *et al.* (1995). The addition of water (approx. 3%) did not reduce any antibacterial property but the addition of edible oil (approx. 3%) reduced this property. This may be due to the miscibility of the active components and oil resulting in the interference of the hydrolysis system. There was no antibacterial activity from shallot even though it is in the same family as garlic. Ankri and Mirelman (1999) detected no alliin, the precusor for allicin, in onion, while Xiao and Parkin (2000) found isoalliin and cyloalliin in onion instead of alliin. Regardless, the Tom Yum mix and kaffir lime leaves have potential for antibacterial effects while some scientific experiments failed to find its antibacterial property by using the disk diffusion method (Mackeen *et al.,* 1997). There was no antibacterial effect in lemon grass, galangal root or chili in this study. Some experiments found capsaicin to be the main active compound for pungency or heat sensation and has an antibacterial property in *Helicobacter pylori* (Jones *et al.,* 1997). However, this compound has a low solubility in water (Santamaria *et al.,* 2000) and that may be the reason why chili has no bacterial effect. Moreover, it is possible that the concentration and/or purity of active compounds in each spice are not enough to inhibit test bacteria. Therefore, antimicrobial properties depend on several factors such as type, composition and concentration of the spices, concentration of target microorganisms and so on (Fung *et al.,* 1985) and even extraction method; as well as forms used, such as essential oil (Fung *et al.,* 1985) or crude extract, as used in this study. However, when lemon grass, kiffir lime leaves, galangal root, shallot, garlic and red chili were combined and made into Tom-Yum mixture, the mix showed antibacterial activities. Some chemical and physical properties of Tom-Yum mix and its ingredient are shown in Table 3; Appendix 2. Garlic, in certain amounts, still has

enough potential to express its antibacterial property, so it could be increased in the formulation without losing consumer acceptance, making Tom-Yum mix more antibacterial. Citric acid, which is normally used for limejuice substitution in a commercial product, also expressed its antimicrobial activity when the concentration was high enough. This is an alternative choice to consider for a new formulation.

Table 11 Antibacterial activities on test bacteria from various samples

Sample	Test Bacteria			
	E. coli O157 : H7	*P. flourescens*	*S. aureus*	*L. monocytogens*
Tom-Yum mix (fresh)	++	-	++	++
Turmaric	-	-	-	-
Shallot	-	-	-	-
Kaffer lime leaves	-	-	+	-
Chili				
Red chili	-	-	-	-
Green chili	-	-	-	-
Seed	-	-	-	-
Galangal	-	-	-	-
Lemon grass	-	-	-	-
Garlic				
Whole garlic	+++	+++	+++	+++
Garlic heart	-	-/ +	-	-
Cooked whole garlic	-	-	-	-
Garlic + Galangal	++	++	++	++
Garlic + water	+++	++	+++	+++
Garlic + edible oil	+	+	+	+
Edible oil	-	-	-	-
3.5% citric acid	- (0)	- (0)	- (0)	- (0)
4.5% citric acid	- (0)	+	+	- (0)
5.5% citric acid	- (0)	++	+ +	++

- no inhibition or clear zone

- (0) there was an inhibition zone but no clear zone

+ there was very small clear zone (dimension < 1.5 cm)

++ there was medium clear zone (dimension >1.5 cm)

+++ there was big clear zone (dimension > 2.0 cm)

β-carotene content

Table 12 shows that chili in its red stage was a main source of β-carotene. The red pulp finger chili itself had the highest β-carotene ($p<0.05$), but it is not used in the original Tom-Yum recipe, where the peak area was very low. Besides the red chili, kaffir lime leaves, particularly in their intermediate or old stage could be a second major source of β-carotene in Tom-Yum mix. Mature leaves contained more β-carotene than young leaves and this supports the findings of Deli *et al.* (2001) and Hornero-Mendez and Minguez-Mosquera (2000), but this contrasted with the results of Deli *et al.* (1996) and Deli and Toth (1997). The amount of β-carotene in chili from this study seemed higher than previous reports, which are in the range of 1.80-159.00 μg/g sample (Bhaskachary *et al.*, 1995; Howard *et al.*, 2000; Hussein *et al.*, 2000) but close to the results of Breithaupt and Bamedi (2001). There are several factors that can affect β-carotene content. There may be species, variety, time of year and ripeness (Deli *et al.*, 2001) and even extraction method including the solvent and saponification step. Some researchers reported that the saponification step yields higher β-carotene content (Minguez-Mosquera and Hornero-Mendez, 1994; Hart and Scott, 1995; Howard *et al.*, 2000) while several papers recently reported that the saponification step causes a lower yield of this compound (Oliver *et al.*, 1998; Granado *et al.*, 2001). No isoflavone was found in any test sample even though the retention time of this was identical to standard daizein and genistein found in legume plants.

Table 12 Summary of β-carotene content in test samples[1]

Sample	β-carotene content
	μg/g sample [2]
Shallot	0.15 ± 0.32F
Garlic (whole)	0.20 ± 0.42F
Garlic (flesh)	0.00 ± 0.00F
Garlic (green heart)	2.51 ± 0.84F
Lemon grass (whole)	3.19 ± 3.67F
Lemon grass (wooden)*	3.10 ± 1.54F
Lemon grass (biggest)**	0.00 ± 0.00F
Lemon grass (leafy)***	0.37 ± 0.34F
Galangal (whole)	0.89 ± 0.24F
Galangal (bark)	0.69 ± 1.20F
Galangal (xylem)	0.00 ± 0.00F
Galangal (dried)	0.00 ± 0.00F
Kaffir lime leaves (medium)	173.60 ± 61.45C
Kaffir lime leaves (young)	78.80 ± 34.06D
Thai chili (green)	12.85 ± 8.17EF
Thai chili (orange)	30.63 ± 9.19E
Thai chili (red)	192.04 ± 139.12B
Finger chili (whole)	204.75 ± 46.72B
Finger chili (pulp)	287.18 ± 36.35A
Tom-Yum (fresh)	2.96 ± 0.72F
Commercial Tom-Yum A	5.09 ± 1.22F
Commercial Tom-Yum B	7.67 ± 1.40F

[1] Based on fresh weight

[2] Each value represents the mean and standard deviation from three lots.

[A-F] Means within a column with a different letter are significantly different ($p<0.05$)

* Plant part above the ground about 1 cm, which is wooded and light yellow

** Plant part above the ground about 2-3 cm, which has the biggest dimension

*** Plant part above the ground about 8-10 cm, which is green and leafy

3.2 Effects of Tom-Yum mix on microbial growth and consumer preference

3.2.1 Effects of Tom-Yum mix on survival of target bacteria

Characteristic of Tom-Yum mix

pH, a_w and color of Tom-Yum mix were 5.4-5.6, 0.89-0.92 light orange to dark orange, respectively.

It was found that the bacterial load of Tom-Yum mix was within the range 10^1-10^3 cfu/ g as shown in Table 13. This implies that those plants, herbs and spices could also be a source of microorganisms. Variation of bacterial counts in each lot may be due to planting conditions, harvesting, and transportation etc. However, the bacterial count decreased to less than 20 cfu/g in later days depending on the initial load. Thus, it could be that active compounds were released and expressed more activity compared with those on the first day, or that bacteria may resist against the active components for a while before a bactericidal effect was expressed. However, if bacterial load in plants is high then antibacterial properties for the test microorganism may be reduced since part of it may be used to combat the contaminated bacteria on the plants themselves. Therefore, sanitary handling of these plant materials is necessary before the antimicrobial properties of Tom-Yum mix can be more accurately determined.

Table 13 Bacterial load (log_{10} cfu/g) of fresh Tom-Yum mix kept at 4 $\pm 1^0$C

Lot no.	Storage time (day)								
	0	1	2	3	4	5	6	7	8
1	2.83	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
2	3.12	3.05	1.91	1.36	1.36	1.36	1.30	1.30	1.30
3	3.18	2.26	1.30	1.30	1.30	1.30	1.30	1.30	1.30
4	1.74	nd	nd	nd	nd	nd	nd	nd	1.30

nd; not determined

The results also showed that *L. monocytogenes* was the most resistant to Tom -Yum mix as shown in Table 14 because its population declined by just 1 log cycle. One reason may be due to its psychrophile property, while *Staphylococcus aureus* was the most sensitive bacteria. This result was similar to the results of Alzamora *et al.* (2000) who reported that gram-positive bacteria are generally more sensitive to phenolic compounds found in plants. Branen *et al.* (1980) reported the inhibitory effect of phenolic compounds against the following bacteria: *Salmonella senftenberg, Samonella typhimurium, Staphyloccus aureus, Clostridium perfringens, Pseudomonas fluorescens and Pseudomonas fragi.* Zaika (1988) also concluded that microorganisms differ in their resistance toward a spice or herb and a given microorganism will differ in its resistance to different spices or herbs. Moreover, food components can affect, increase (by the presence of acids, humectants, antimicrobial, etc.,) or reduce (by partitioning of active components into the lipid phase, etc.,) the antimicrobial capacity. The antimicrobial efficiency of a spice or herb depends on its origin, handling, processing, and storage. Raccach (1984) reviewed the literature on the antimicrobial activity of phenolic compounds and mentioned that its activity appeared to be strongly dependent on microorganisms in the spices (strain and concentration); type and concentration of phenolic antioxidants; combination of phenolic antioxidants; combination with other antimicrobials; temperature; and food additives and components. The concentration of phenolic antioxidants with antimicrobial activity was in the range of 30-10,000 ppm, whereas these compounds are permitted as antioxidants in concentrations generally up to 200 ppm, based on the fat or oil content of the food product. From our observations, it was found that at high serial dilution (low bacterial count) *S. aureus* would lose its pigment intensity from deep yellow to pale yellow before it died at that concentration later. This may imply that some active components in Tom-Yum mix are involved in pigment production and the death process. Ruiz-Barba *et al.* (1990), using scanning electron microscopy, showed that cells without treatment were smooth compared with those treated with phenols for 24 hr, which appeared rugged and with irregular surface. *P. fluorescens* and *E. coli* O157 : H7 were quite easily

destroyed in the mix compared with results from previous experiments as was determined by the disk diffusion method. This may be that co-culture could provide more contact time, more active compounds, or the penetration process could occur easily. Some investigators found that the disk diffusion method is a qualitative method while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are quantitative methods. So, the disk diffusion method should be used as an antibacterial screening step. However, if there is any attempt to use Tom-Yum mix as a natural preservative then it should be tested with a food system to confirm this antibacterial activity.

Table 14 Effects of Tom-Yum mix on survival of test bacteria (log_{10} cfu /ml) cultured in peptone water

Test bacteria	Incubation time (day)								
	Trt	0	1	2	3	4	5	6	7	8
S. aureus	1	9.1	9.2	9.2	9.3	9.1	9.0	9.0	9.1	9.0
	2	9.2	8.6	7.4	7.2	6.8	6.3	5.6	4.6	3.7
E.coli O157:H7	1	9.4	9.3	9.1	9.3	9.2	9.1	9.1	9.2	9.1
	2	9.6	7.8	7.5	6.3	5.1	5.4	5.1	4.7	4.5
P.fluorescense	1	9.2	9.1	9.3	9.0	9.1	9.1	9.0	9.0	9.0
	2	9.2	7.9	7.3	6.2	6.5	6.5	5.5	5.2	4.8
L.monocytogeneses	1	9.3	9.6	9.7	9.6	9.5	9.5	9.1	9.1	9.1
	2	9.4	9.6	9.5	9.4	9.3	9.3	9.0	9.0	8.2

Note: Trt: Treatment, 1: control sample (without Tom-Yum), 2: culture with Tom-Yum.
In high decimal dilution (low bacterial load) of *S. aureus*, the result found that there is loss of the characteristic of bacteria such as pale colony.

3.2.2 Effects of Tom-Yum mix formulas on consumer preference

Characteristics of Tom-Yum mix

pH, a_w and color of Tom-Yum mix were 5.4-5.7, 0.89-0.92 light orange and dark orange, respectively. This is similar to the previous experiment described in section 3.1.

For spice flavor intensity, there was only one panelist among the 26 people who could identify that garlic flavor increased as garlic content in formulation increased, as shown in Table 15. Most panelists preferred the Tom-Yum soup that contained more garlic than less garlic. This signifies a potential of using garlic in the recipe. As the results confirmed, the garlic flavor did not have a negative effect on certain Tom-Yum formulations (ie. score did not exceed 3), even though some home-style recipes would not favor adding garlic. The results also showed that formulations B and C obtained higher consumer preference than other formulations even though the score was just over "neither like nor dislike". The panelists' opinion was that the taste was still mild and not as strong as what they are familiar with. This may be because the panelists in this experiment were Thais who prefer soup with a strong taste and flavor. However, adding more Tom-Yum mix when cooking or marinating should solve this problem.

Like garlic flavor intensity, the other ingredients were low in their flavor intensity, so it can be predicted that most consumers around the world will accept the formulated Tom-Yum mix, as this Tom-Yum mix was tested on several people in the United States of America (unpublished data). Even if people in some countries prefer to add more or less of some ingredients, this should not be a problem since they can vary the amounts of the ingredients according to taste.

The results showed that Tom-Yum mix could reduce the population of target bacteria within range 1-4 log cycles depending on the type of bacteria. The most resistant bacterium in the mix was *L. monocytogenes*. However, antibacterial properties should be improved by adding a greater amount of garlic without fear of a customer preference problem. Either formula B or C can be used as a fish marinating model in the further step. In section 4, formula C was used for marinating fish.

Table 15 Flavor intensity and consumer preference of various Tom-Yum formulas

Attribute	Formula			
	A	B	C	D
	Flavor intensity			
Garlic flavor	2.30	2.47	2.57	3.07
Shallot flavor	2.23	2.43	2.33	2.80
Galangal flavor	2.50	2.50	2.57	3.13
Lemon grass flavor	2.07	2.23	2.17	2.60
Kaffir lime leaves	2.63	2.70	2.53	3.20
	Taste intensity			
Hot of chili	2.27	2.47	2.30	2.33
Consumer preference	2.67	3.27	3.23	2.87

A : basic formula

B : 2 times garlic content of basic formula

C : 3 times garlic content of basic formula

D : 4 times garlic content of basic formula

4. Shelf-life extension of cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres

4.1 Screening for suitable gas mixture for cut fish marinated with selected Tom-Yum mix formula

pH changes

As a whole, it was found that pH of fish flesh decreased as a concentration of CO_2 increased as shown in Fig. 14. This is due to carbonic acid and/or lactic acid accumulation owing to the retardation of some specific bacteria producing amine. Lannelongue *et al.* (1982) also reported that pH in CO_2-enriched packaging groups was lower than in the control group due to CO_2 absorption and dissolution in moisture at its tissue surface in the first four days. Thereafter it tended to increase. pH value of the control group tended to increase as storage time increased due to amine compounds produced from both endogenous and exogenous enzymes (Lannelongue *et al.*, 1982). Regardless of the control group, there was no significant difference ($p>0.05$) in pH between the groups treated with different gas mixtures. Moreover, it was found that pH tended to decrease as storage time increased. This may be due to the effect of an accumulation of carbonic acid and lactic acid as mentioned above. This result was similar to other researchers who reported that the changes in pH were dependant on the amount of dissolved CO_2 and buffering capacity of the tissue (Banks *et al.*, 1980; Lanneolongue *et al.*, 1982; Ruiz-Capillas and Moral, 2001 a, b; Ruiz-Capillas *et al.* 2002). These authors have also stated that in the samples stored in a modified atmosphere with different CO_2 concentrations there is a lower production of basic substances most likely because of lower microbial activity.

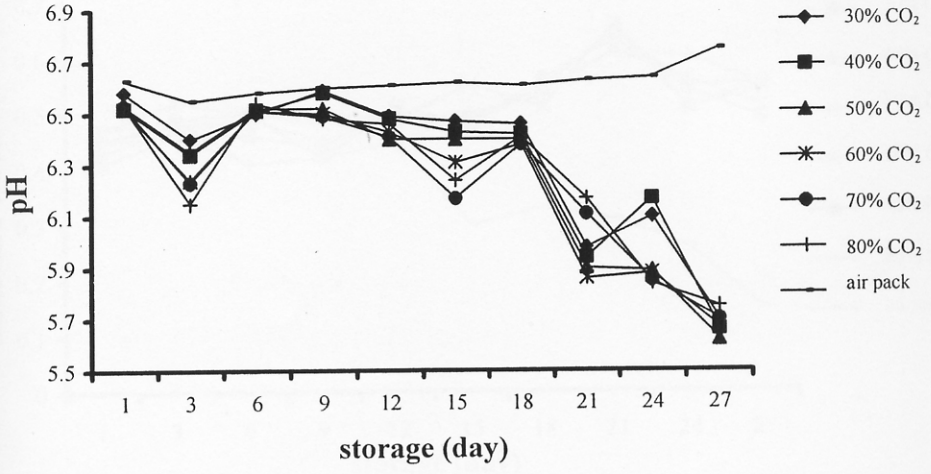

Fig. 14 pH changes in cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres during storage 4 ±1°C

Lactic acid content

It was found that lactic acid content in each group increased as storage time increased except in the control group as shown in Fig. 15. This may be because modified atmosphere packaging enhances lactic acid bacteria growth and also carbonic acid accumulation may be involved. This result agreed with the report of Nassos *et al.* (1983). However, Drosinos and Board (1995) reported that lactic acid content decreased as storage time increased, but the condition of storage consisted of high O_2, which was not a good condition for lactic acid bacteria, facultative or microaerobic bacteria. In this experiment O_2, fixed at 5%, may provide a suitable condition for lactic acid bacteria. Thus, this was possibly why the result was different.

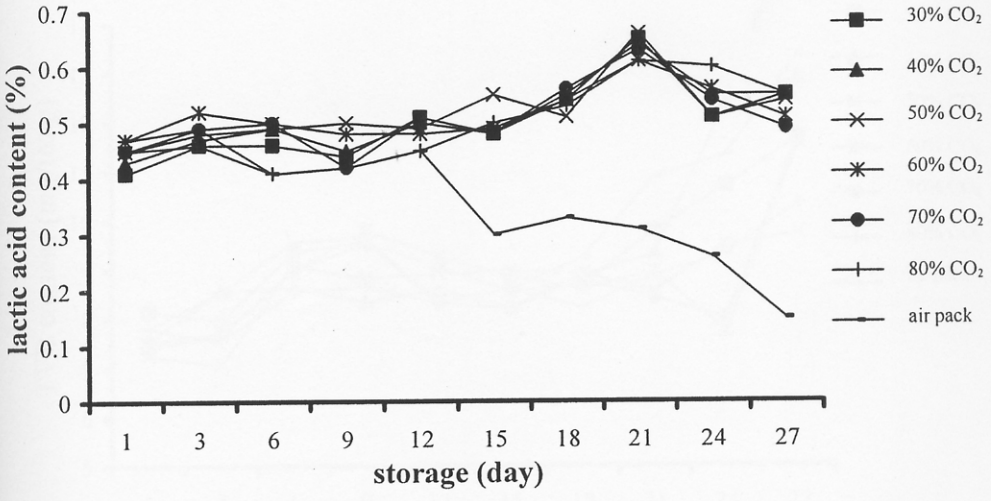

Fig. 15 Lactic acid content in cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres during storage at 4 ±1°C

TVB changes

As a whole, TVB tended to increase as storage time increased, particularly in control groups as shown in Fig. 16. However, this value was under the standard limit of 25-35 mg /100 g samples, (Botta, 1995) throughout the storage time even if the fish showed spoilage signs. This agreed with reports of several published papers that have mentioned an absence of non-linear correlation between length of time (day) and fish spoilage (Botta *et al.*, 1984; Storey *et al.*, 1984; Connell *et al.*, 1986; Howgate, 1986; Kolakowski, 1986; Perez-Villarrea and Howgate, 1987). However, all samples marinated with Tom-Yum Mix and kept under a modified atmosphere could retard the bacterial growth and enzymatic activity. This result agreed with Lannelongue *et al.* (1982) who reported the slower rate of TVB production in a CO_2-enriched atmosphere, which could be due to a reduction in the development of psychrotropic aerobic bacteria.

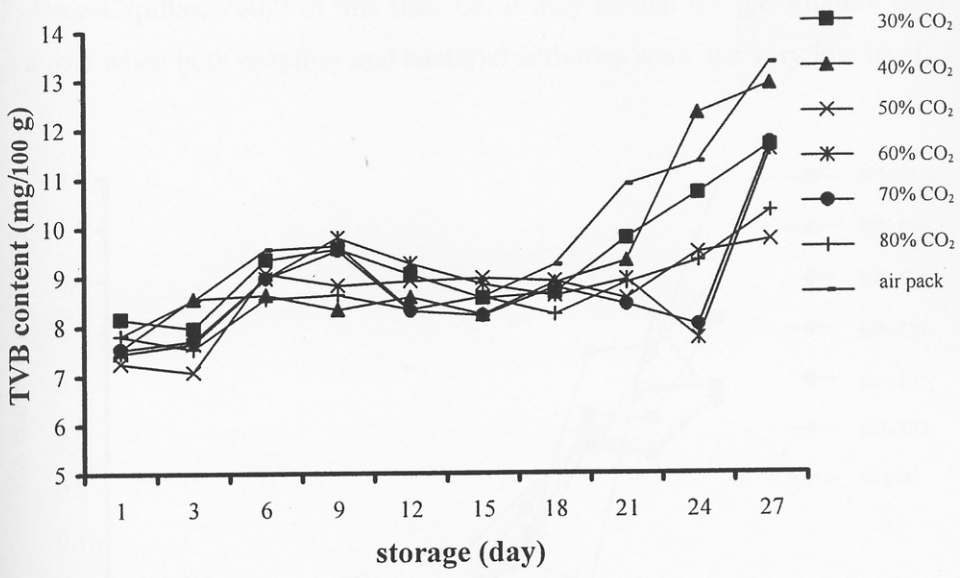

Fig. 16 TVB content in cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres during storage at 4 ±1°C

TMA changes

The TMA in samples treated with Tom-Yum mix and packaged under a modified atmosphere occurred after day 15 while in the control group it was apparent as early as day 12 of storage as shown in Fig. 17. This result implied that quality loss in the control group is faster than in the treated groups due to greater bacterial activity. This was similar to the results of Ben-gigirey *et al.* (1999). However, the TMA value in this fish was low and was lower than the standard limit, even in the control group, possibly due to the nature of the fish. Seabass is brackish water fish not sea water fish. Therefore, TMAO, substrate for TMA production, was either absent or very low. Moreover, even if TMAO is distributed among all kinds of marine and invertebrates, and in some fresh water fish, TMAOase activity has been identified in very few marine fish (Sikorski and Kostuch, 1982). Rehbein and Schreiber (1984) stated that there are a lot of factors affecting TMAOase such as gender, maturation stage, temperature of the habitat, feeding status, size as well as seasonal variation (Gill, 1982). However, it may be possible that it was present only at low levels in gram-negative psychrotrophic bacteria producing TMAOase (Lannelongue *et al.*,

1982; Ruiz-Capillas, 2002) in this fish. Or, it may be that the gas mixture ratio also plays a role when both enzymes and bacterial activities were at a very low level.

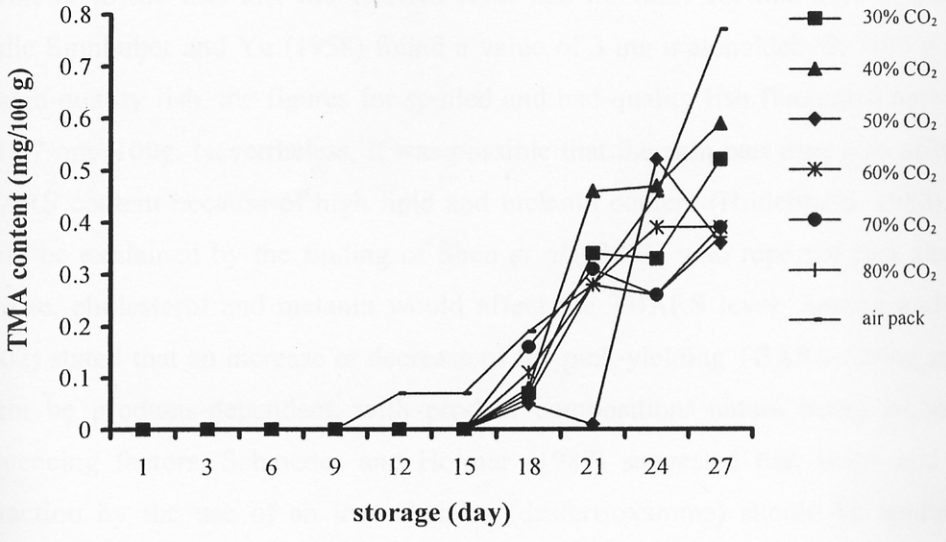

Fig. 17 TMA content in cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres during storage at 4±1°C

TBARS changes

As shown in Fig. 18, there were large variations of TBARS content found in every fish sample. This was different from previous reports in which TBARS increased as storage time increased. Recently, however, some researchers have agreed that TBARS is not a good indicator for detecting oxidized products because malonaldehyde, the main compound can also interact with protein, peptide, lipid compounds as well as change into other compounds (Ruiz-Capillas *et al.*, 2002). Moreover, Gross *et al.* (1994), Jamora and Rhee (2002), concluded that the TBARS assay was not a suitable measure for lipid oxidation. Ben-gigrey *et al.* (1999) found that although the TBARS levels for the frozen albacore tuna were acceptable they indicated slight rancidity. In this experiment, it was found that TBARS tended to increase to a certain level and then decrease before increasing again. This result was similar to the results of Ben-gigirey *et al.* (1999). However, at high concentrations of the CO_2 group, TBARS level was higher than other groups having an earlier storage time. MacDonald and Hultin (1987) reported that lipid oxidation is affected by pH,

lipid composition, ionic strength, temperature, redox potential, light exposure and iron content. However, from our observations, there was no rancid odor in any marinated treatment throughout the storage, which may be due to Tom-Yum mix masking the flavor, or to the fact that the TBARS level had no value for this kind of product. While Sinnhuber and Yu (1958) found a value of 3 mg malonaldehyde /100 g tissue in high-quality fish, the figures for spoiled and bad-quality fish fluctuated between 4 and 27 mg/ 100g. Nevertheless, it was possible that the skin part may also affect the TBARS content because of high lipid and melanin content (Hildebrand, 1988). This could be explained by the finding of Sheu *et al.* (2003) who reported that albumin, sucrose, cholesterol and melanin would affect the TBARS level. Jamora and Rhee (2002) stated that an increase or decrease of the pink-yielding TBARS during storage might be products-dependent, with product composition/ nature being among the influencing factors. Schmedes and Holmer (1987) suggested that Bligh and Dyer extraction by the use of an iron chelator (desferrioxamine) should be applied for TBARS determination in cod tissue. The TBARS test could determine both the free malonaldehyde already formed naturally from hydroperoxide cleavage, and the secondary release due to the heating step in TBA reaction. Therefore, the TBA values obtained are dependent on methodology and not easily reproduced between the laboratories (Schmedes and Holmer, 1987). From our observations, it was found that variation in TBARS content might be affected by extracting muscle with acid solution because the blending step may increase air content resulting in higher lipid oxidation, coinciding with results obtained from Lee *et al.* (1999). To avoid this problem, pulverizing the samples with a pestle and mortar should be introduced.

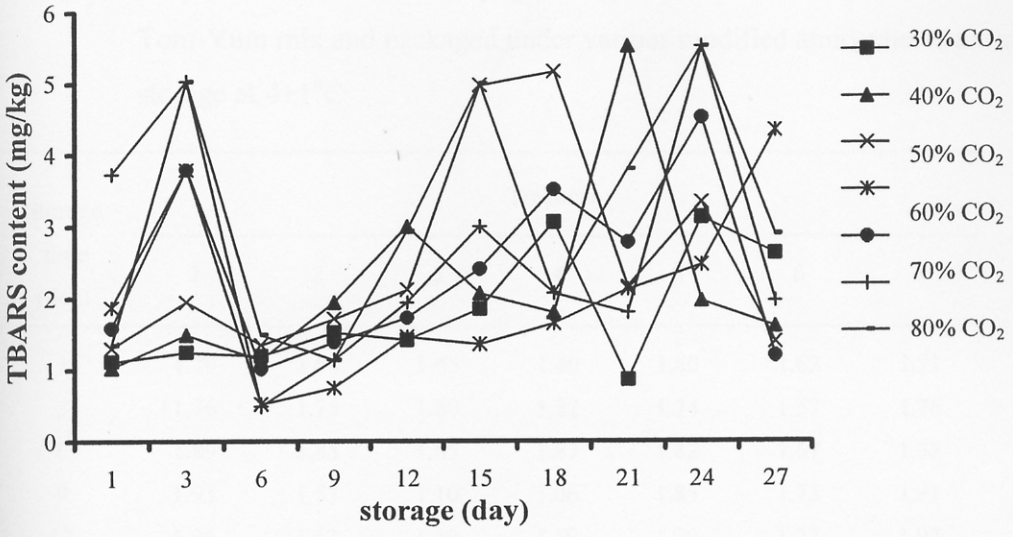

Fig. 18 TBARS content in cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres during storage at 4 ±1°C

Mesophilic bacteria

It was clear that marinating fish with Tom-Yum mix and packaged under a modified atmosphere could better retard bacterial growth than in the control as shown in Table 16. Standard bacterial count is limited to about 10^6-10^7 cfu/g. The more concentration of CO_2 the less bacterial growth occurred. Both carbonic acid and lactic acid can cause the death of some bacteria by its weak acid. Compared with the control group it was found that fish marinated with Tom-Yum mix could extend the shelf life from 12 days up to 18 to 27 days, depending on the concentration of CO_2. However, in the next experiment marinating with Tom-Yum mix and packaging under a modified atmosphere could be used for another control to confirm whether the extension of shelf-life would be affected by the modified atmosphere and/or the Tom-Yum mix.

Table 16 Mesophilic bacteria counts (log_{10} cfu/g) in cut fish marinated with Tom-Yum mix and packaged under various modified atmospheres during storage at 4±1°C

Storage time (day)	Treatment						
	1	2	3	4	5	6	7
1	1.79	1.68	1.65	1.40	1.60	1.62	1.71
3	1.76	1.75	1.80	1.82	1.74	1.57	1.76
6	1.89	1.85	1.85	1.87	1.82	1.67	1.82
9	1.93	1.53	1.10	1.06	1.85	1.75	1.91
12	1.96	1.53	1.59	1.59	1.90	1.73	1.97
15	1.98	1.70	1.43	1.56	1.91	1.81	2.13
18	2.48	2.09	1.81	1.52	1.94	1.70	2.81
21	2.85	2.09	2.1	2.23	1.95	1.79	nd
24	nd	nd	2.27	nd	2.20	1.97	nd
27	nd	nd	nd	nd	nd	2.08	nd

(1) 30%CO_2: 65%N_2: 5%O_2, (2) 40%CO_2: 60%N_2: 5%O_2, (3) 50%CO_2: 45%N_2: 5%O_2
(4) 60%CO_2: 35%N_2: 5%O_2, (5) 70%CO_2: 25%N_2: 5%O_2, (6) 80%CO_2: 15%N_2: 5%O_2
(7) air pack, nd: not determined

4.2 Effects of Tom-Yum mix on shelf-life of fish packaged under 70 to 90% CO_2 atmospheres

pH changes

It was found that the CO_2-enriched packaging had a pH of 6.15-6.17 while pH of the control was 6.20 as shown in Fig. 19. This might be due to carbonic acid accumulation, which coincided with the result of Ruiz-Capillas. (2001 b, c). The pH in treated samples tended to slightly decrease on day 3 and then remained at around 6.03-6.04. This is similar to the result of Lyhs *et al.* (2001) who reported that vacuum-packaged "gravid" (sugar-salted fish products) rainbow trout had an initial pH of about of 6.49 and slightly changed to 6.37 and 6.29 at the time of spoilage at 3°C and 8°C of storage, respectively. In the control group, pH tended to increase as storage time increased and reached pH 6.49. The pH changes pattern was similar to the previous experiments even when the figures were lower, which may be due to the different lots.

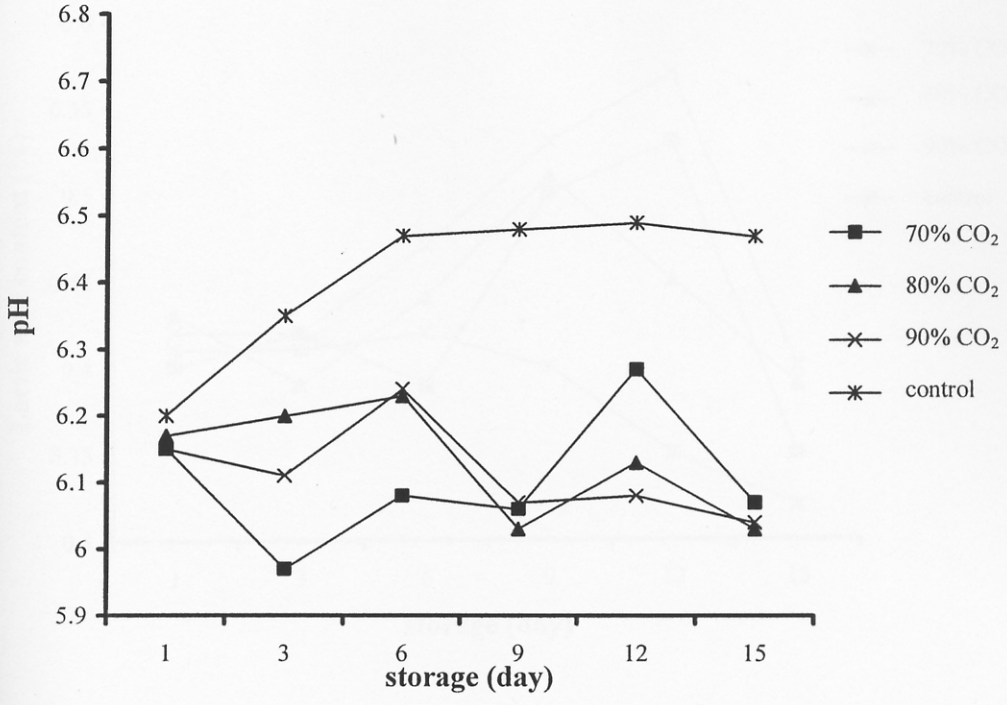

Fig. 19 pH changes in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Lactic acid content

Lactic acid content was shown in Fig. 20. The contents correlated well with pH values (R=0.6-0.8). However, it should be recognized that the pH value in this experiment was not only produced from lactic acid, but also carbonic acid provided by CO_2 absorption and dissolution, as well as other acids. In the control sample, it was found that lactic acid decreased as storage time increased, coinciding with the results of the previous experiments and those of other researchers (Drosinos and Board, 1995).

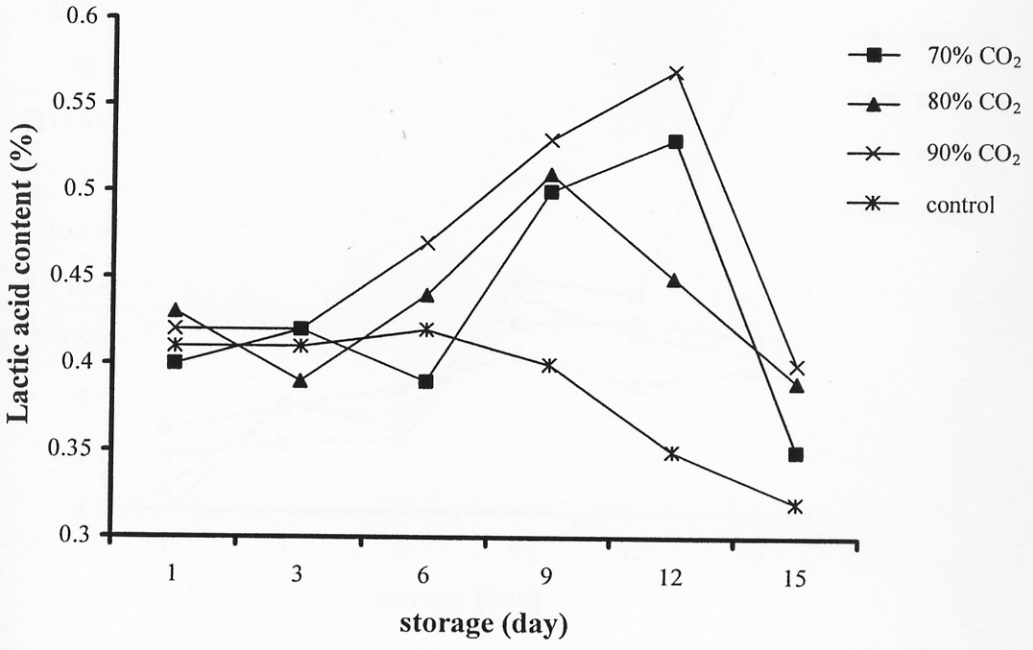

Fig. 20 Lactic content in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

TVB changes

The data from Fig. 21 shows that TVB increased as storage time increased. Although there were some differences ($p<0.05$) among the groups, the value was lower than the standard limit in all treatments. This agreed with the previous study. However, the control sample tended to have a higher TVB content than fish marinated with Tom-Yum and packaged under a modified atmosphere. This is because there was no Tom-Yum mix to act as a barrier against microbial growth.

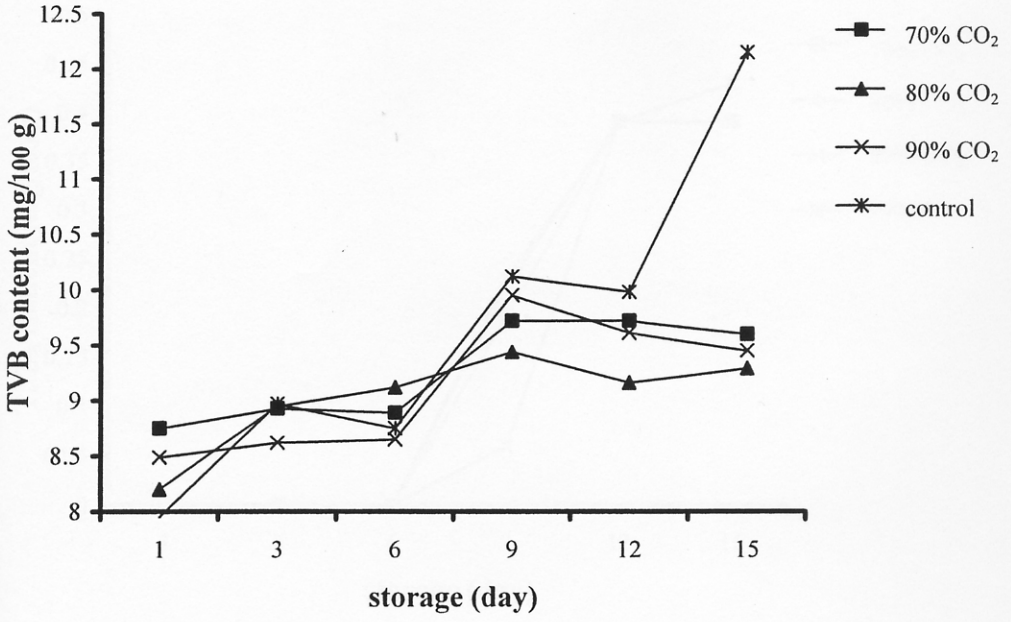

Fig. 21 TVB content in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

TMA changes

Similar to previous study, TMA in all fish samples was very low as shown in Fig. 22. There was no TMA in any of the samples in the first 6 days of chilled storage. This result confirmed that TMA in seabass would not reach the standard limit (3-4 mg/100 g sample). One of the reasons could be the type and load of bacteria contained in the fish. Nevertheless, this experiment was also done under controlled conditions, which did not allow for much bacterial contamination. Thus, any sign of spoilage cannot be determined by using this value.

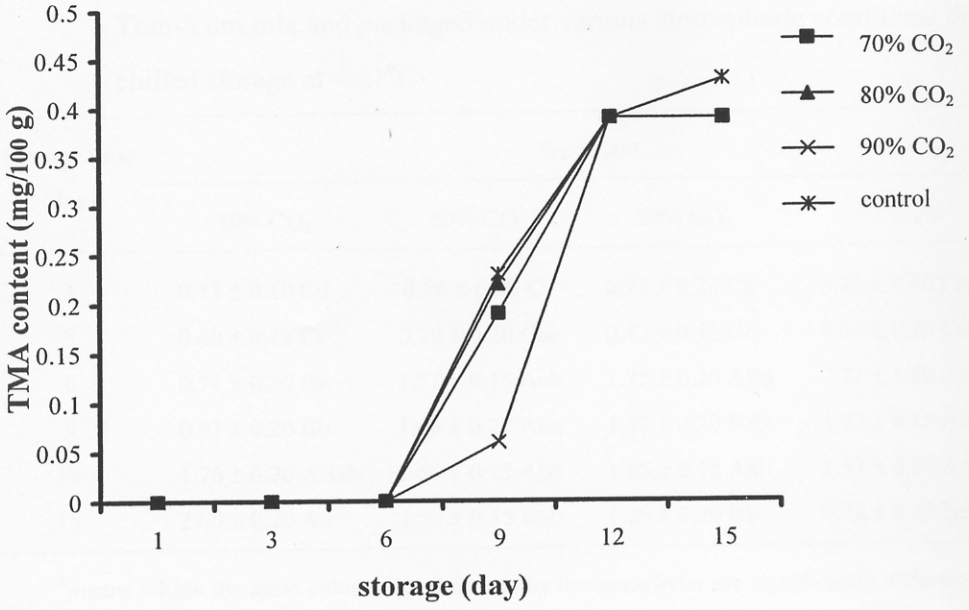

Fig. 22 TMA content in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

TBARS content (mg MDA/kg sample) at absorbance 532

Regardless of the control group, it was found that the higher CO_2 concentration resulted in higher TBARS content as shown in Table 17. This might an effect of metmyoglobin action of cell leakage from carbonic acid, which enhanced lipid oxidation (Lee *et al.*, 1999). Moreover, at concentrations 80% and 90% of CO_2, TBARS content tended to increase as storage time increased, but then decreased, which agreed with the previous study. With a higher concentration of CO_2, TBARS increased as storage time increased, which can be the effect of high acid concentration. Since this storage was just for 15 days it may not account for the whole process of some treatment as compared with the previous experiments. The higher TBARS content in the control group may be due to lack of antioxidant agent from Tom-Yum mix such as β-carotene or other phenolic compounds, and also high O_2 content. Removal of the skin may help reduce variation of TBARS level compared with the previous study (screening for suitable gas mix). Lee *et al.* (1999) found that contamination of melanin also interfered with TBARS content.

Table 17 TBARS content (mg MDA/kg sample) 532 nm in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	70% CO_2	80% CO_2	90% CO_2	control
1	0.13 ± 0.10 Cd	0.58 ± 0.20 Cb	0.73 ± 0.20 Ca	0.23 ± 0.10 Cbc
3	0.40 ± 0.15 Cb	0.79 ± 0.20 Cac	0.43 ± 0.15 Cb	0.54 ± 0.20 Cab
6	0.71 ± 0.20 Bb	1.71 ± 0.15 Aab	1.73 ± 0.20 ABa	2.31 ± 1.20 Aa
9	0.91 ± 0.20 Bb	1.66 ± 0.20 Aba	1.37 ± 0.20 Bab	1.43 ± 0.15 Abab
12	1.76 ± 0.20 ABab	1.66 ± 0.15 Abb	1.95 ± 0.15 Aab	2.57 ± 0.20 Aa
15	2.00 ± 0.20 Aa	1.56 ± 0.15 Bab	1.26 ± 0.20 Bb	0.78 ± 0.20 Bc

[A]means within the same column not followed by the same letter are significantly different

[a]means within the same row not followed by the same letter are significantly different.

TBARS content (mg MDA/kg sample) at absorbance 450

Table 18 shows that in addition to the pink-yielding pigment, yellow to orange pigment was also involved in the lipid oxidation process. However, no correlation could be made between pink and yellow pigment. Therefore, other analytical methods such as HPLC and GC/LC-MS may be needed to determine the changes of the exact compounds and its effects before solid conclusions can be reached.

Table 18 TBARS content (mg MDA/kg sample) at 450 nm in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	70% CO_2	80% CO_2	90% CO_2	control
1	0.23 ± 0.10 Ba	0.31 ± 0.20 Ca	0.32 ± 0.15 Ca	0.15 ± 0.10 BCa
3	0.36 ± 0.20 Bb	0.92 ± 0.15 Aa	0.37 ± 0.20 BCb	0.23 ± 0.15 ABb
6	0.47 ± 0.20 Abab	0.59 ± 0.20 ABa	0.55 ± 0.20 Aa	0.13 ± 0.10 Bb
9	0.39 ± 0.15 Bab	0.41 ± 0.20 Ba	0.41 ± 0.15 Ba	0.27 ± 0.10 ABb
12	0.46 ± 0.20 Abab	0.61 ± 0.20 ABa	0.55 ± 0.20 Aa	0.32 ± 0.10 Ab
15	0.57 ± 0.20 Aab	0.73 ± 0.15 Aa	0.39 ± 0.20 Bb	0.09 ± 0.05 Cc

[A]means within the same column not followed by the same letter are significantly different.

[a]means within the same row not followed by the same letter are significantly different.

Microbiological analyses

Mesophilic bacteria

It was found that mesophilic bacteria content in all treated samples was less than 10^6 cfu/g (< 6 log $_{10}$ cfu/g) throughout the storage time as shown in Fig. 23. The control group just reached the 10^6 cfu/g at the end of the storage period. This result disagreed with previous studies. The lower figure in mesophilic bacterial count in this experiment as compared to previous ones may be due to the different bacterial type contained in each fish lot. Moreover, it was also possible that Tom-Yum mix, with a high concentration of CO_2, and kept in low storage temperature may reduce or retard the bacterial growth. In general, CO_2 has a more pronounced antibacterial property on gram-negative bacteria than gram-positive bacteria because of their different cell structures. It is known that there are many factors affecting antibacterial property such as initial bacterial load, type of bacteria, ingredients of the food system, and pH (Parish and Davidson, 1993). Therefore, the use of different raw material lots may also provide differences in results.

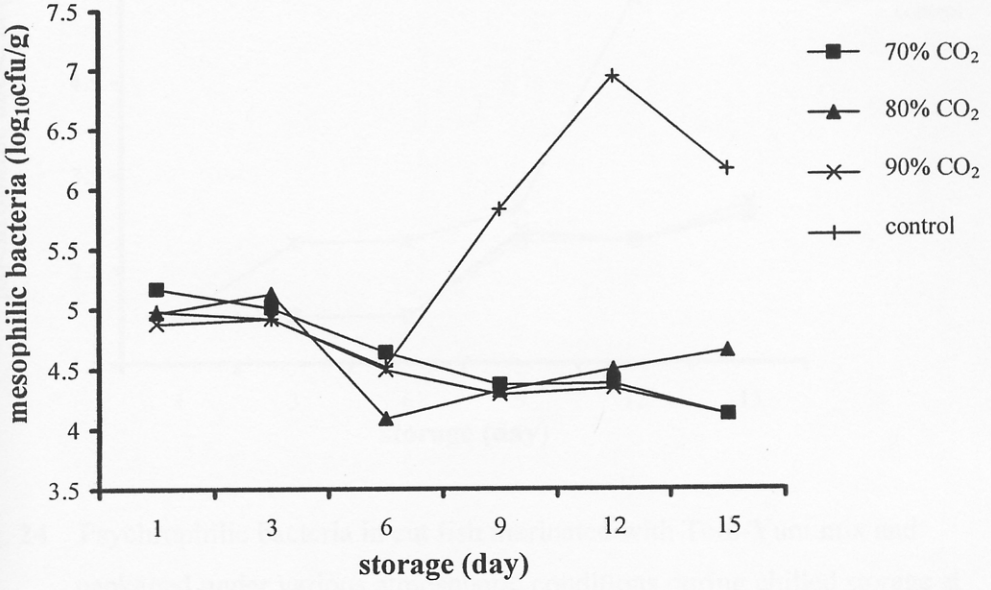

Fig. 23 Mesophilic bacteria in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Psychrophilic bacteria

In general, no significant difference in psychrophilic bacterial count occurred in any of the treated samples ($p>0.05$) as shown in Fig. 24, even on samples from different days. Thus fish marinated with Tom-Yum Mix and kept under concentrations of CO_2 of around 70 to 90% at low storage temperature provided effective organism inhibition. This may be due to the synergistic effect of stable active compounds and more CO_2 absorption of tissue at low temperatures. Moreover, the nature of tropical fish such as seabass, and special care in sample handling may also contribute to synergizing the inhibitive effect. Regarding the control group, at the end of storage, bacterial count reached 10^6 cfu/g as expected. This may be due to the absence of any barrier Tom-Yum mix that might provide defense against psychrophilic bacterial growth. Therefore, psychrophilic bacterial growth plays an important role in chilled fish spoilage.

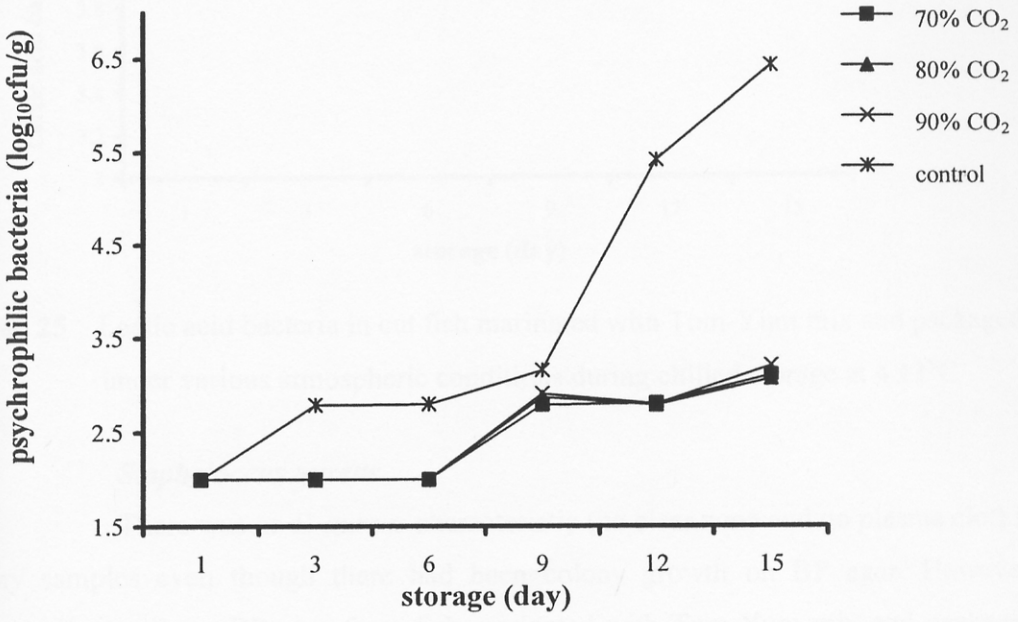

Fig. 24 Psychrophilic bacteria in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Lactic acid bacteria

Amounts of lactic acid bacteria are shown in Fig. 25. There were no significant differences ($p>0.05$) between in any of the samples. This evidence was very surprising as generally, lactic acid bacteria were major bacteria found in vacuum packaged or modified atmosphere packaged products. However, some researchers reported that even in normal air packaging, CO_2 led to micro aerobic condition, which in turn led to lactic acid bacteria growth. This caused the acid odor in the normal air package. However, it may be possible that MRS was not a specific enough agar to differentiate lactic acid bacteria from other bacteria that can grow in the MSR agar.

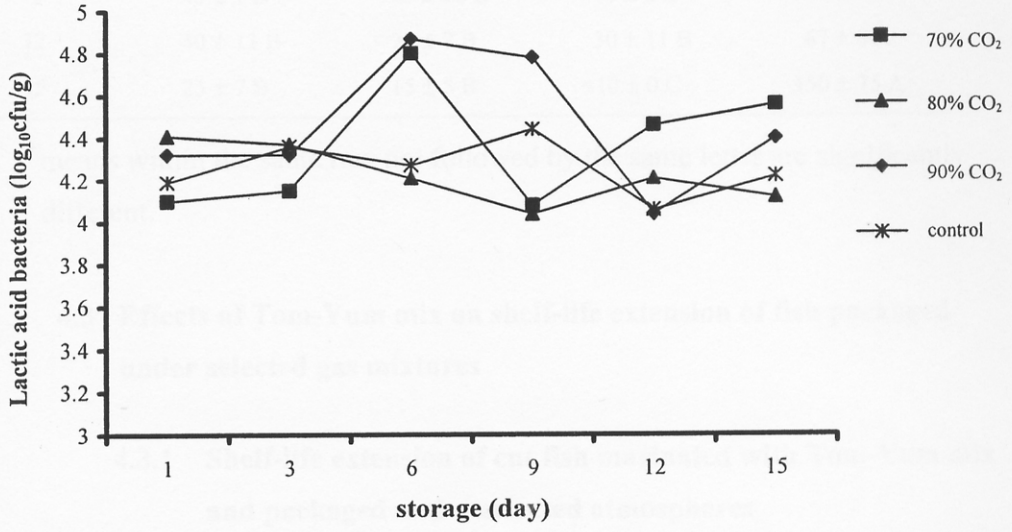

Fig. 25 Lactic acid bacteria in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Staphyloccus aureus

There was no *S. aureus* characteristic (no clear zone and no plasma clot) in any samples even though there had been colony growth on BP agar. However, bacteria growth on BP agar from fish marinated with Tom-Yum mix and packaged under modified atmospheres was lower than in the control group as shown in Fig. 19. Moreover, it was found that the higher the concentration of CO_2 the lower the bacterial count. Regarding antibacterial properties of Tom-Yum mix, it was found that *S. aureus* was the most sensitive bacteria in comparison with other test bacteria. Thus, it may be one of the reasons why treated samples had a low level of this bacterium. Another possibility could be the highly sanitized handling conditions, which prevented the growth of *S. aureus*.

Table 19 None-clear-zone-producing *Staphylococcus aureus* (cfu/g) in cut fish marinated with Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	70% CO_2	80% CO_2	90% CO_2	control
1	40 ± 10 A	30 ± 15 A	30 ± 17 A	60 ± 20 A
3	60 ± 30 A	35 ± 27A	60 ± 13 A	222 ± 18 A
6	35 ± 15 A	45 ± 21B	25 ± 23 A	60 ± 32 A
9	45 ± 7 B	25 ± 20 B	10 ± 5 C	125 ± 70 A
12	40 ± 13 B	20 ± 7 B	30 ± 11 B	67 ± 8 A
15	25 ± 7 B	15 ± 5 B	<10 ± 0 C	350 ± 75 A

[A]means within the same row not followed by the same letter are significantly different.

4.3 Effects of Tom-Yum mix on shelf-life extension of fish packaged under selected gas mixtures

4.3.1 Shelf-life extension of cut fish marinated with Tom-Yum mix and packaged under selected atmospheres

pH changes

As shown in Fig. 26, in the first few days, there was no significant difference to be found in the treatment and control samples. However, pH in the control group without Tom-Yum mix (C_2) tended to have a higher value than other groups ($p<0.05$) after samples were kept for 12 days. pH in samples treated with Tom-Yum mix and or CO_2–enriched packaging (T_1, T_2 and C_1) remained stable throughout the storage time. This result was different from the previous studies as well as from the results of Bremner and Statham (1987) but agreed with the results of Lannelongue *et al.* (1982), and Layrisse and Matches (1984). However, all samples had pH values lower than 7, which implied that the total volatile nitrogen concentration was still low. According to Wang and Brown (1983), the increase in pH is associated with bacterial growth and is probably caused by the formation of basic

amines. Stansby and Olcott (1963) questioned the reliability of pH values as a freshness test because acidic and basic by-products of spoilage tend to neutralize each other. Thus, other types of analysis are needed to evaluate the freshness of fish. These results show that pH changes could differ from batch to batch, which makes pH value unreliable as a determination of fish quality change.

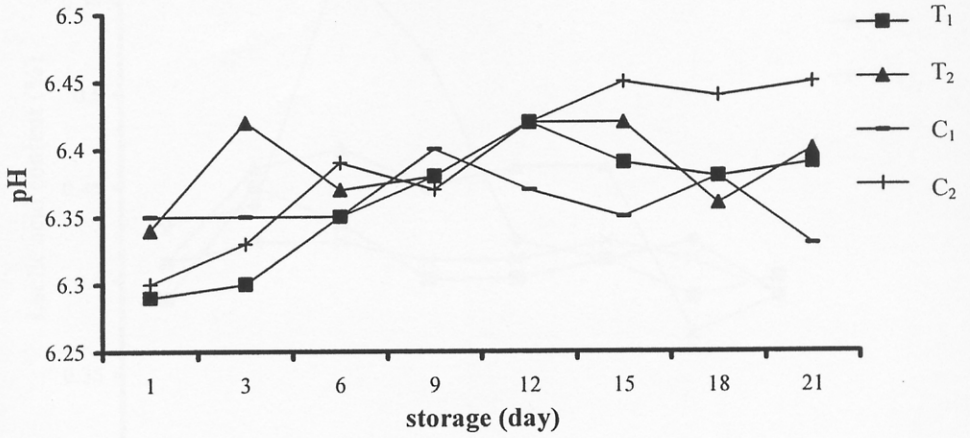

Fig. 26 pH changes in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

Lactic acid content (%)

The results show that lactic acid content tended to increase slightly as storage time increased, then decreased in later days as shown in Fig. 27, except in the control group, which coincided with the results of previous research (Kakouri and Nychas, 1994). However, in the few samples in which *Brocchothrix thermophacta* dominated rather than lactic acid bacteria, increases in L-lactic acid were recorded (Drosinos and Board, 1995). It could be implied that accumulation of acid is microbial dependent. In this experiment, however, it could imply that both carbonic acid (Bremner and Statham, 1987) and lactic acid (Nassos *et al.*, 1983; Drosinos and

Board, 1995) were the main factors affecting the acidity in fish muscle. However, the decrease of lactic acid or total acid may be due to the process of neutralization with volatile base nitrogen (Stansby and Olcott, 1963).

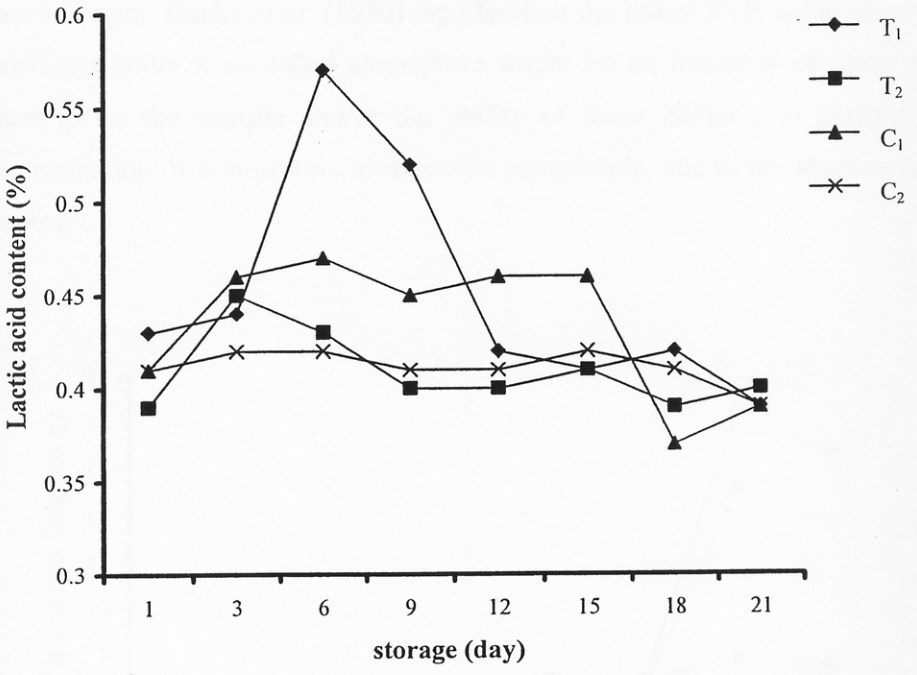

Fig. 27 Lactic acid content in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2 :5%N_2 :5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

TVB changes

The results show that treatment T_1 and C_1 (both marinated with Tom-Yum mix) seemed to have higher TVB than the treatment that had no added Tom-Yum mix, as shown in Fig. 28. It may be that the treatment treated with Tom-Yum mix had more initial load and variety of bacteria. However, after 15 days of storage the control group (C_2) (without marinating with Tom-Yum mix and kept in normal air) had the

highest TVB content in comparison with other groups ($p<0.05$). This agrees with the results of Villemure *et al.* (1986). The TVB content in the treatments T_1, T_2 and C_1 remained stable throughout the storage time. Therefore, it can be deduced that both Tom-Yum mix and CO_2-enriched packaging could reduce bacterial producing volatile base nitrogen. Banks *et al.* (1980) reported that the lower TVB value observed in fish packaged under a modified atmosphere might be an indicator of lower number of bacteria on the sample and/or the ability of those bacteria to perform oxidative determination of non-protein nitrogenous compounds, due to the absence of O_2 in the package.

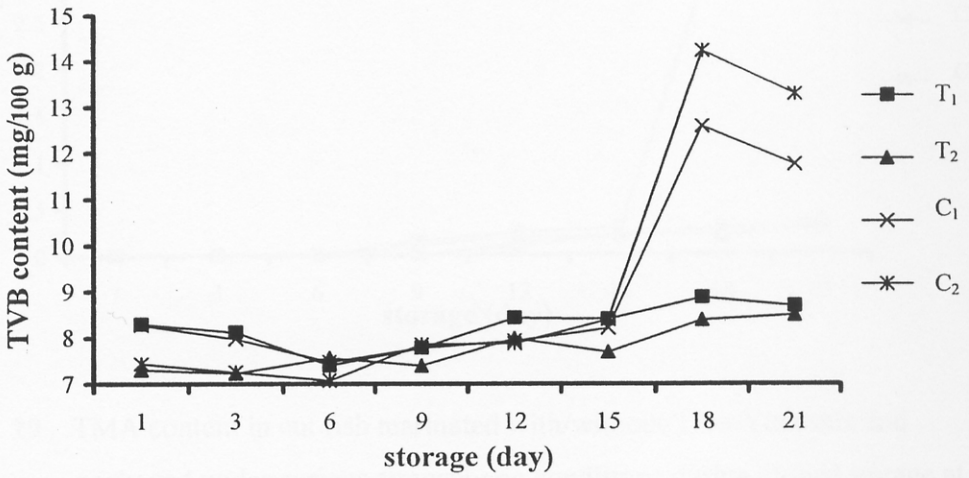

Fig. 28 TVB content in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at $4 \pm 1^{\circ}C$

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

TMA changes

The results show that TMA produced from TMAO via TMAOase affected the fish treated with Tom-Yum mix but not that of CO_2-enriched packaging in later days as shown in Fig. 29. This may be due to the antibacterial activities from Tom-Yum mix as reported in previous studies. The TMA value increased as storage time increased but it remained lower than the standard limit throughout the storage time. However, in the control group that had no Tom-Yum mix (C_2), the TMA value was 3.46 ± 0.40 on day 18.

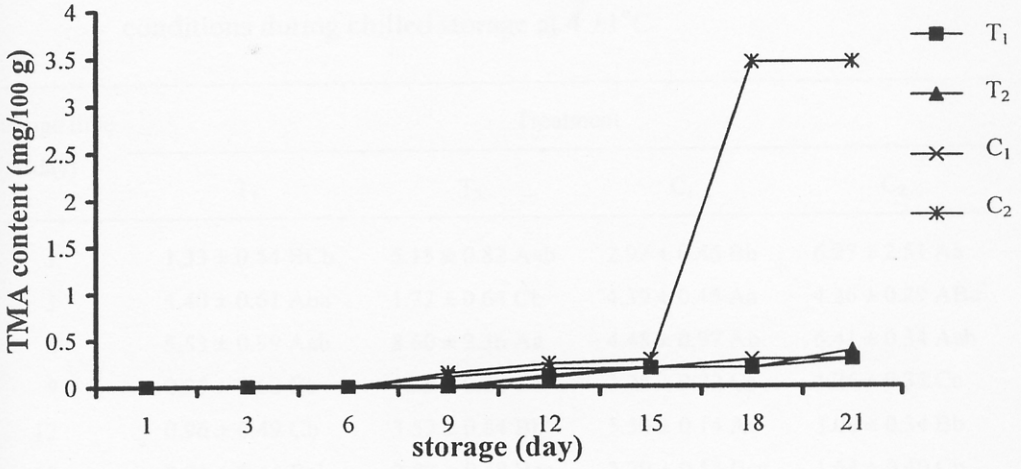

Fig. 29 TMA content in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

TBARS content (mgMDA/kg sample) at absorbance of 532 nm

As shown in Table 20, it was found that TBARS value changed continually throughout the storage time as occurred in the previous study. This also agreed with the results of Bak *et al.* (1999). Fish muscle is not a homogeneous sample; this may be an important factor affecting the variation of TBARS value. Therefore, another indicator is needed to measure lipid oxidation more accurately.

Table 20 TBARS content (mgMAD/kg sample) at 532 nm in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	T_1	T_2	C_1	C_2
1	1.33 ± 0.54 BCb	5.15 ± 0.82 Aab	2.92 ± 0.46 Bb	6.27 ± 2.51 Aa
3	4.40 ± 0.61 Aba	1.77 ± 0.64 Cb	4.39 ± 0.45 Aa	4.26 ± 0.29 ABa
6	5.53 ± 0.99 Aab	8.60 ± 2.36 Aa	4.48 ± 0.97 Ab	6.41 ± 0.34 Aab
9	0.25 ± 0.62 Cb	1.38 ± 0.34 Ca	1.75 ± 0.22 Ca	1.26 ± 0.35 Ca
12	0.96 ± 0.49 Cb	3.52 ± 0.84 Bb	5.50 ± 0.14 Aa	3.06 ± 0.34 Bb
15	2.21 ± 0.44 Bab	2.87 ± 0.48 Bca	2.79 ± 0.53 Bca	1.64 ± 0.40 Cb
18	1.85 ± 0.22 BCbc	2.61 ± 0.83 Cb	4.71 ± 2.26 Aa	1.43 ± 0.42 Cc
21	2.90 ± 1.67 Bab	3.00 ± 0.36 Ba	2.47 ± 0.90 Bca	1.25 ± 0.53 Cb

[A]means within the same column not followed by the same letter are significantly different

[a]means with in the same row not followed by the same letter are significantly different

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

TBARS content (mg MDA/kg sample) at absorbance of 450 nm

It was found that oxidized lipid product measured at an absorbance of 450 nm changed continually throughout the storage as shown in Table 21. The results are similar to TBARS values measured at an absorbance of 532 nm. Therefore, TBARS values measured at both wavelengths were not a good indicator for lipid oxidation for this product. This TBARS value seemed to be product and/or storage

time dependent. This also agreed with many researchers who found that an increase or decrease of the TBARS value during storage was product-dependent with product composition/nature being among the influencing factors (Jamora and Rhee, 2002). Moreover, it was found that garlic provided the deepest orange pigment (data not shown) while the other ingredients also gave the pigment in the range of pink to orange color. This finding implied that some compounds in plants interfered in the TBARS value, for example, chlorophyll, and ascorbic acid (at a certain concentration) (Lin and Liang, 2002) and should be investigated further.

Table 21 TBARS content (mg/kg sample) at 450 nm in cut fish marinated with/ without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	T_1	T_2	C_1	C_2
1	0.40 ± 0.08 Cc	0.52 ± 0.07 ABb	0.61 ± 0.05 BCab	0.73 ± 0.34 Aa
3	0.91 ± 0.03 Ab	0.11 ± 0.12 Cc	1.21 ± 0.11 Ba	0.75 ± 0.03 Abc
6	0.70 ± 0.22 ABb	0.42 ± 0.14 ABc	0.91 ± 0.27 BCa	0.64 ± 0.20 Ab
9	0.00 ± 0.00 Ea	0.00 ± 0.00 Da	0.00 ± 0.00 Ca	0.00 ± 0.00 Da
12	0.19 ± 0.14 Db	0.17 ± 0.11 Cb	0.90 ± 0.27 BCa	0.17 ± 0.06 C
15	0.48 ± 0.07 Bb	0.42 ± 0.11 Bb	1.37 ± 0.46 Ba	0.31 ± 0.02 Bc
18	0.22 ± 0.01 Dc	0.73 ± 0.87 Aab	1.94 ± 1.17 ABa	0.55 ± 0.38 ABb
21	0.59 ± 0.11 Bb	0.50 ± 0.04 ABb	4.76 ± 1.60 Aa	0.35 ± 0.06 Bc

[A] means within the same column not followed by the same letter are significantly different

[a] means with in the same row not followed by the same letter are significantly different

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

Mesophilic bacteria

Mesophilic bacteria increased as storage time increased as shown in Fig. 30. The bacterial counts in day 1 were in the range 3.8 to 5.6 $\log_{10}$ cfu/g. Using the standard bacterial count (10^6 cfu/g (Boerema *et al.*, 1993)) as a guide it was found

that treatment T_1, T_2, C_1 and C_2 had a shelf-life of 18, 15, 15 and 12 days, respectively. These results indicated that a combination of Tom-Yum mix and CO_2-enriched atmosphere packaging is more effective in controlling bacterial growth than either Tom-Yum mix or CO_2-enriched atmosphere packaging alone. This may be due to the fact that CO_2 caused injury to bacterial cells. Therefore, it appeared that active compounds such as phenolic compounds, allicin etc., from Tom-Yum mix were expressing their antibacterial activities. However, when compared with results of the screening for gas mixture section, it was found that the fish sample in this experiment had a shorter shelf-life. This different result may be due to two major reasons; the first was initial bacterial load both in Tom-Yum mix and fish fillet, and the second reason was quantity and quality of active compounds in Tom-Yum mix ingredient. Lopez-Caballero *et al.* (1999) stated that both the initial microbial population and the composition of spoilage microflora influenced the stability of vacuum-packed ham. Farid, *et al.*, 1998 reported that catfish fillet shelf-life strongly depended on the initial microbiological quality.

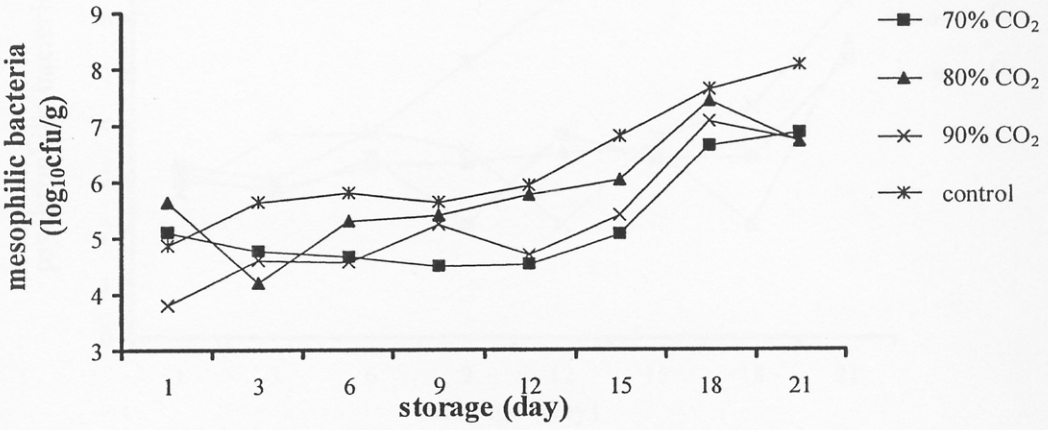

Fig. 30 Mesophilic bacteria in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at $4 \pm 1^{\circ}C$

T_1 : marinated with Tom-Yum mix and kept under $90\%CO_2$: $5\%N_2$: $5\%O_2$

T_2 : without marinating with Tom-Yum mix and kept under $90\%CO_2$: $5\%N_2$: $5\%O_2$

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

Psychrophilic bacteria

It was found that treatments using Tom-Yum mix and packaged under CO_2-enrichment (T_1) had a higher inhibition effect on psychrophilic bacteria as shown in Fig. 31. Therefore, the psychrophilic bacterial counts in treatment T_1 remained lower than the other treatments throughout the storage time; except for treatment T_2 and C_1. This result could imply that phychrophilic bacteria are more sensitive to both Tom-Yum mix and high concentrations of CO_2 rather than either one alone. Without Tom-Yum mix or CO_2-enriched atmosphere packaging (C_2), psychrophilic bacteria tended to increase as storage time increased. This agreed with the results of Newsome *et al.* (1984) and Al-Dagal and Bazaraa (1999) who reported that psychrophilic bacteria growth is a function of storage time.

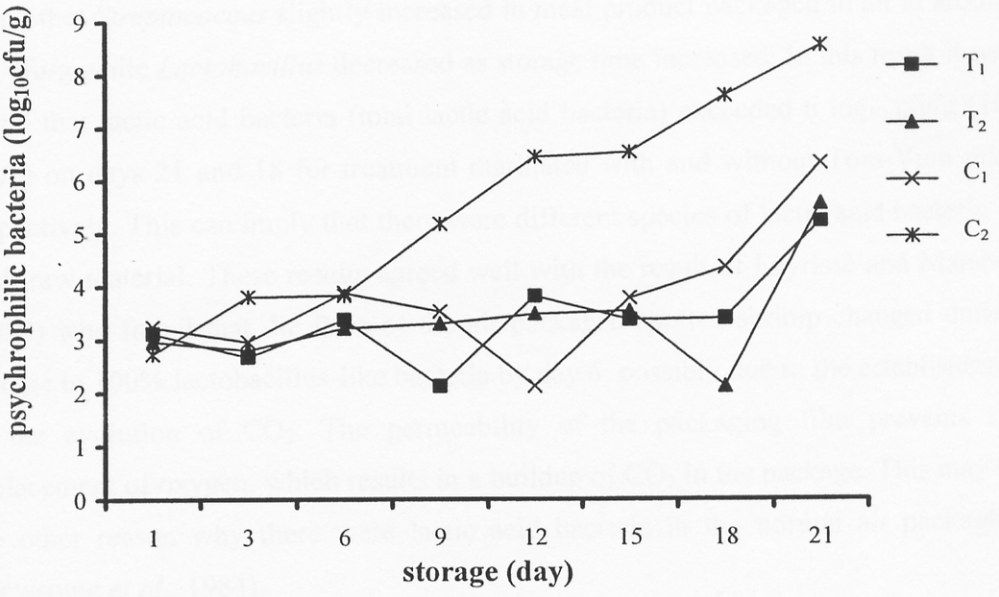

Fig.31 Psychrophilic bacteria in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom Yum-mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

Lactic acid bacteria

Results on lactic acid bacteria are shown in Fig. 32. These results agree with the previous studies that lactic acid bacteria occurred in all samples. It was also found that even in Tom-Yum mix, there were 10^3 cfu/g lactic acid bacteria (data not shown). However, it was surprising that in treatment C_2, there were more lactic acid bacteria and these bacteria tended to increase as storage time increased. Some researchers stated that in MAP, the majority of bacteria were lactobacilli while there were various amounts of lactic acid bacteria in normal air packaging. The different results may be due to the nature of raw material containing different varieties and quantities of bacteria. Ringo and Strom (1994) reported that while the population level of lactobacilli decreased, counts of *Leuconostoc* spp. and *Streptococcus* spp. remained stable when the Arctic charr were reared in seawater. Newsome *et al.* (1984) found that *Streptococcus* slightly increased in meat product packaged in air at around 10^4 cfu/g while *Lactobacillus* decreased as storage time increased. In this result it was found that lactic acid bacteria (total lactic acid bacteria) exceeded 6 $\log_{10}$ cfu/g (10^6 cfu/g) on days 21 and 18 for treatment marinated with and without Tom-Yum mix, respectively. This can imply that there were different species of lactic acid bacteria in each raw material. These results agreed well with the result of Layrisse and Matches (1984) who found that the flora of the air packaged spotted-shrimp changed during storage to 100% lactobacillus-like bacteria by day 6, possibly due to the establishment of the evolution of CO_2. The permeability of the packaging film prevents the replacement of oxygen, which results in a buildup of CO_2 in the package. This may be the other reason why there were lactic acid bacteria in the normal air packaging (Newsome *et al.*, 1984).

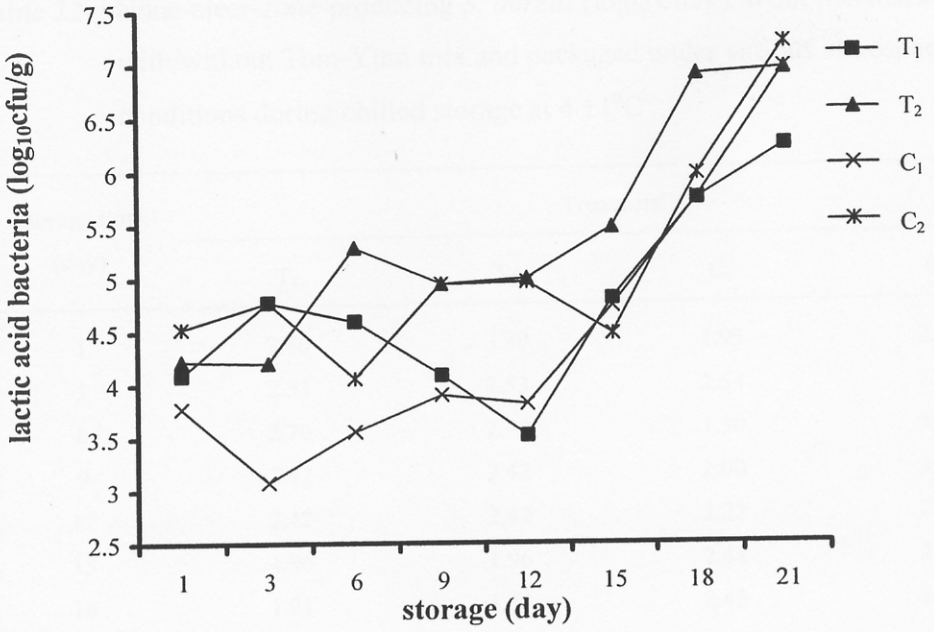

Fig. 32 Lactic acid bacteria in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

S. aureus

It was found that marinating fish with Tom-Yum mix and keeping under 90%CO_2: 5%N_2: 5%O_2 had a strong effect on normal *Staphylococcus aureus* bacteria after fish were stored for 6 days as shown in Table 22. The control sample (C_2) had higher none-clear-zone-producing *Staphylococcus* than any other samples. These results agreed with the previous studies and confirmed that Tom-Yum mix still expressed antibacterial activities and also had a synergistic effect with CO_2-enriched packaging in the real food system. Therefore, this signifies a high potential use for Tom-Yum mix in industrialized systems.

Table 22 None-clear-zone-producing *S. aureus* (log_{10} cfu/g) in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	T_1	T_2	C_1	C_2
1	3.80	3.80	1.95	2.29
3	2.53	2.53	2.54	2.83
6	2.70	2.70	1.30	3.71
9	2.42	2.42	2.00	3.51
12	2.42	2.42	2.22	2.70
15	1.96	1.96	2.48	2.48
18	1.81	1.81	2.42	3.61
21	1.48	1.48	1.00	6.58

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2:5%N_2:5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2:5%N_2:5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

Coliform bacteria

It was found that coliform bacteria occurred in all samples in the first 6 days (data not shown). These bacteria tended to decrease as storage time increased except in control group C_2. However, there was no *E. coli* in any of the samples. This may be due to good sanitation of culturing water. In addition, seabass used in this experiment were cultured in cases in Songkhla Lake, and not in the pond as some provinces have been doing, thus the moving water system in Songkhla Lake may help sanitize the fish. Moreover, marinating it with Tom-Yum mix and keeping it in a high concentration of CO_2 could also help retard bacterial growth as explained in the review section.

Sensory evaluation

Table 23-24 show that the sample treated with Tom-Yum mix had a higher flavor score, particularly when compared with control group (C_2). This may be due to the spice flavor. The good flavor in marinated Tom-Yum mix occurred after fish were kept for 9 days. However, there were two negative side effects of marinating fish with Tom-Yum mix and keeping it under a high concentration of CO_2. These were a bit sour taste and soft dry texture, which may be due to protein degradation from acid caused by the use of high concentrations of CO_2 in the package (Cheftel and Cheftel, 1976; Villuemure *et al.*, 1986; Bak *et al.*, 1999) reported that the toughness was significantly higher for shrimp packed in normal air compared to shrimp packed in modified air. However, after cooking, there was no significant difference in any treatment even in the control group (C_2). Judging from sensory evaluation, all samples had a shelf-life of more than 18 days.

Table 23 Sensory evaluation intensity in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage (day) \ Treatment	Attribute	T_1	T_2	C_1	C_2
1th day	Acid	1.82 ± 1.13	1.41 ± 0.87	1.71 ± 0.92	1.22 ± 0.43
	Fishy	2.11 ± 1.20	3.00 ± 1.24	1.89 ± 1.37	3.06 ± 1.00
	Rancid	1.50 ± 0.71	1.72 ± 0.88	1.50 ± 0.80	1.89 ± 1.23
	Good smell	3.17 ± 1.06	1.89 ± 1.06	3.17 ± 0.86	2.22 ± 0.94
	Spice	3.39 ± 0.87	1.11 ± 0.39	3.22 ± 1.12	1.50 ± 0.99
	Sour	1.17 ± 0.44	1.17 ± 0.44	1.28 ± 0.61	1.28 ± 0.67
	Sweet	2.11 ± 0.97	2.22 ± 1.11	1.72 ± 0.73	2.17 ± 1.10
	Ubami	2.17 ± 0.85	2.22 ± 1.17	2.00 ± 0.99	2.33 ± 1.19
	Brown	2.17 ± 0.96	1.72 ± 1.19	2.44 ± 1.37	1.47 ± 0.62
	Pink	1.33 ± 1.03	1.33 ± 0.87	0.94 ± 0.35	1.41 ± 0.62
	Grey	1.67 ± 0.81	1.78 ± 1.27	1.83 ± 1.20	1.82 ± 0.73
	Cream	2.44 ± 1.44	2.78 ± 1.43	2.11 ± 1.25	3.53 ± 1.07
	Mashy	1.89 ± 1.78	1.94 ± 1.14	2.06 ± 1.33	2.59 ± 1.12
	Dry tough	2.06 ± 1.30	1.89 ± 1.17	2.00 ± 0.99	1.88 ± 0.86
	Tough	3.11 ± 0.63	2.89 ± 1.25	2.39 ± 1.46	2.71 ± 1.40
6th day	Acid	1.71 ± 0.85	1.31 ± 0.79	1.75 ± 1.34	1.41 ± 0.73
	Fishy	2.41 ± 1.42	3.00 ± 1.32	2.25 ± 1.18	2.88 ± 1.11
	Rancid	1.82 ± 1.19	1.63 ± 0.89	1.63 ± 1.02	1.94 ± 1.32
	Good smell	3.12 ± 1.17	1.88 ± 1.02	3.38 ± 1.36	2.00 ± 1.29
	Spice	2.82 ± 1.33	1.38 ± 0.72	3.38 ± 1.26	1.18 ± 0.40
	Sour	1.47 ± 0.51	1.19 ± 0.54	1.44 ± 0.63	1.24 ± 0.34
	Sweet	1.94 ± 0.66	2.13 ± 1.15	2.25 ± 0.77	2.18 ± 1.17
	Ubami	2.06 ± 0.75	2.50 ± 1.00	2.60 ± 1.00	2.40 ± 1.40
	Brown	2.82 ± 1.33	1.38 ± 0.72	3.20 ± 1.32	2.25 ± 1.11
	Pink	1.24 ± 0.56	1.25 ± 0.45	1.07 ± 0.26	1.50 ± 0.52
	Grey	2.00 ± 1.17	1.88 ± 1.09	2.00 ± 1.13	1.81 ± 0.86
	Cream	2.00 ± 1.00	3.69 ± 1.35	2.47 ± 1.06	2.75 ± 1.19
	Mashy	2.29 ± 1.16	2.69 ± 1.25	2.13 ± 0.99	2.25 ± 0.96
	Dry tough	2.00 ± 1.06	1.94 ± 0.77	2.20 ± 0.77	1.94 ± 0.85
	Tough	2.82 ± 1.33	2.56 ± 1.26	2.87 ± 1.06	2.69 ± 0.83

Table 23 Sensory evaluation intensity in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C(cont.)

Storage time \ Treatment	Attribute	T_1	T_2	C_1	C_2
12th day	Acid	1.48 ± 0.68	1.32 ± 0.67	1.68 ± 0.95	1.68 ± 0.95
	Fishy	2.00 ± 1.14	2.58 ± 1.02	1.58 ± 0.84	1.58 ± 0.84
	Rancid	1.24 ± 0.54	1.58 ± 1.07	1.21 ± 0.71	1.21 ± 0.71
	Good smell	3.19 ± 1.03	2.21 ± 1.27	3.68 ± 0.95	3.58 ± 1.02
	Spice	3.95 ± 0.74	1.53 ± 1.26	4.16 ± 1.17	4.16 ± 1.17
	Sour	1.38 ± 0.63	1.11 ± 0.32	1.53 ± 0.70	1.53 ± 0.70
	Sweet	1.90 ± 0.59	2.37 ± 0.32	2.37 ± 0.32	1.74 ± 0.73
	Ubami	1.80 ± 0.83	2.11 ± 1.07	1.95 ± 0.73	1.95 ± 0.71
	Brown	2.00 ± 1.00	1.58 ± 1.10	2.79 ± 0.71	2.79 ± 1.18
	Pink	3.76 ± 1.04	1.37 ± 0.96	1.32 ± 1.18	1.32 ± 0.58
	Grey	1.24 ± 0.54	1.84 ± 0.60	2.11 ± 0.58	2.11 ± 1.20
	Cream	1.71 ± 0.72	3.84 ± 1.30	2.47 ± 1.17	2.53 ± 1.17
	Mashy	2.19 ± 1.12	2.53 ± 1.02	1.84 ± 0.69	1.79 ± 0.71
	Dry tough	1.95 ± 1.40	1.89 ± 0.81	2.63 ± 0.96	2.53 ± 0.90
	Tough	2.90 ± 1.18	2.26 ± 0.87	3.16 ± 1.01	3.16 ± 1.01
18th day	Acid	1.42 ± 0.61	1.37 ± 0.68	1.63 ± 0.83	1.26 ± 0.73
	Fishy	168 ± 0.89	2.58 ± 1.46	2.00 ± 1.33	3.00 ± 1.25
	Rancid	1.26 ± 0.73	1.53 ± 1.02	1.58 ± 0.90	1.58 ± 0.84
	Good smell	3.21 ± 1.08	2.21 ± 0.98	2.89 ± 1.37	2.05 ± 1.08
	Spice	3.63 ± 1.12	2.16 ± 1.50	3.21 ± 1.40	1.37 ± 0.68
	Sour	1.42 ± 0.77	1.37 ± 0.83	1.42 ± 0.61	1.16 ± 0.50
	Sweet	1.79 ± 1.23	2.11 ± 0.94	1.74 ± 0.93	2.37 ± 1.18
	Ubami	1.89 ± 0.74	2.16 ± 0.90	1.89 ± 0.88	2.79 ± 1.16
	Brown	3.95 ± 1.31	2.37 ± 1.38	4.11 ± 1.29	1.42 ± 0.69
	Pink	1.11 ± 0.32	1.26 ± 0.65	1.37 ± 0.68	1.37 ± 0.60
	Grey	1.53 ± 0.77	1.74 ± 0.93	1.42 ± 0.77	1.84 ± 1.01
	Cream	1.95 ± 1.18	3.32 ± 1.70	1.89 ± 1.24	3.84 ± 1.30
	Mashy	1.63 ± 0.76	2.21 ± 1.03	2.11 ± 1.29	2.84 ± 1.30
	Dry tough	2.53 ± 1.31	2.11 ± 1.15	2.79 ± 1.08	1.95 ± 0.78
	Tough	1.42 ± 0.61	1.37 ± 0.68	1.63 ± 0.83	1.26 ± 0.73

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2:5%O_2
T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2:5%O_2
C_1 : marinated with Tom-Yum mix and kept under normal air
C_2 : without marinating with Tom-Yum mix and kept under normal air

Table 24 Sensory evaluation score using 9-point hedonic scale in cut fish marinated with /without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage day \ Treatment	Attribute	T_1	T_2	C_1	C_2
1th day	Flavor	6.28 ± 1.22a	4.33 ± 1.66b	6.00 ± 1.73a	5.44 ± 2.04a
	Taste	5.78 ± 1.22a	4.72 ± 1.84ab	4.72 ± 1.7ab	4.56 ± 2.15b
	Texture	5.28 ± 1.57b	6.06 ± 1.23a	5.61 ± 1.48ab	5.17 ± 2.12b
	Color	4.94 ± 1.93b	5.44 ± 1.64ab	5.56 ± 1.73ab	5.78 ± 2.13a
	Appearance	5.39 ± 1.24a	5.17 ± 1.50a	5.33 ± 1.41a	5.22 ± 0.43a
6th day	Flavor	5.47 ± 1.62ab	4.81 ± 2.01a	6.56 ± 1.55a	5.47 ± 1.67ab
	Taste	5.41 ± 1.62a	5.75 ± 1.18a	5.44 ± 1.50a	5.35 ± 2.19a
	Texture	5.47 ± 1.28b	6.13 ± 1.50a	6.25 ± 1.29a	6.18 ± 1.78a
	Color	5.53 ± 1.12b	5.69 ± 1.62a	5.75 ± 2.02a	5.94 ± 1.71a
	Appearance	5.47 ± 1.62a	5.75 ± 1.57a	5.94 ± 1.44a	5.82 ± 1.93a
12th day	Flavor	6.33 ± 1.15a	5.63 ± 1.71b	6.95 ± 1.58a	5.84 ± 1.57ab
	Taste	6.62 ± 1.56a	5.68 ± 1.57ab	5.42 ± 1.89b	5.21 ± 1.81b
	Texture	5.33 ± 1.93b	5.79 ± 1.96a	5.89 ± 1.37a	5.79 ± 1.36a
	Color	5.76 ± 1.55b	6.47 ± 1.74a	5.58 ± 1.46b	5.42 ± 1.39b
	Appearance	5.48 ± 1.57a	5.32 ± 0.67a	5.68 ± 0.95a	5.21 ± 1.23b
18th day	Flavor	6.21 ± 1.13a	5.11 ± 1.76c	5.42 ± 1.87b	5.16 ± 2.03c
	Taste	5.00 ± 1.49c	5.21 ± 1.72b	5.42 ± 1.74ab	5.95 ± 1.22a
	Texture	5.84 ± 1.34b	6.11 ± 1.15a	5.53 ± 1.68b	6.21 ± 1.47a
	Color	5.79 ± 1.36ab	6.00 ± 1.60a	5.11 ± 1.88b	6.00 ± 1.64a
	Appearance	5.89 ± 1.41a	5.95 ± 1.51a	5.79 ± 1.62a	6.05 ± 1.65a

[a]means within in the same row not followed by the same letter are significantly different

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

4.3.2 Survival of inoculated *S. aureus* and *E. coli* in cut fish marinated with Tom-Yum mix and packaged under selected atmospheres

It was found that both *S. aureus* and *E. coli* were retarded by Tom-Yum mix and $C0_2$-enrichment except in the control group (C_2) as shown in Table 25 and Table 26, respectively. This may be due to the effect of high concentrations of CO_2 and Tom-Yum mix. Therefore, it may be interpreted that they have a synergist effect on bacterial growth. However, antibacterial effects of both CO_2–enriched atmosphere and the Tom-Yum mix should be more pronounced if the initial loads of both bacteria were not too high as in this experiment. Alzamora *et al.* (2000) stated that initial load of microorganisms can affect antimicrobial activities of phenolic compounds. This phenomenon may be explained as an imbalance between active compounds and bacterial counts.

Table 25 Survival of *S. aureus* (log_{10} cfu/g) in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Treatment			
	T_1	T_2	C_1	C_2
1	7.40 ± 6.48	7.18 ± 6.85	7.60 ± 6.00	7.49 ± 6.48
3	7.71 ± 6.48	7.32 ± 6.30	7.54 ± 6.70	7.99 ± 6.60
6	7.72 ± 6.30	7.37 ± 6.60	7.79 ± 6.78	8.13 ± 6.00
9	7.62 ± 6.48	7.86 ± 6.48	7.94 ± 6.78	7.73 ± 6.48
12	7.32 ± 6.60	7.54 ± 6.60	7.49 ± 6.48	7.83 ± 6.70
15	7.08 ± 7.00	7.11 ± 6.48	7.32 ± 6.30	8.05 ± 6.48
18	7.49 ± 6.48	7.43 ± 6.30	7.41 ± 6.48	8.18 ± 6.60
21	7.48 ± 6.30	7.57 ± 6.48	7.63 ± 6.85	nd

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2: 5%N_2: 5%O_2

T_2 : without marinating with Tom-um mix and kept under 90%CO_2: 5%N_2: 5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air

nd : not determined

Table 26 Survival of *E. coli* (log_{10} cfu/g) in cut fish marinated with/without Tom-Yum mix and packaged under various atmospheric conditions during chilled storage at 4 ±1°C

Storage time (day)	Survival of *E.coli* (log_{10} cfu/g)			
	T_1	T_2	C_1	C_2
1	7	7	7	7
3	7	7	7	7
6	7	7	7	7
9	7	7	7	7
12	7	7	7	7
15	7	7	7	7
18	7	7	7	8
21	7	7	7	8

T_1 : marinated with Tom-Yum mix and kept under 90%CO_2:5%N_2:5%O_2

T_2 : without marinating with Tom-Yum mix and kept under 90%CO_2:5%N_2:5%O_2

C_1 : marinated with Tom-Yum mix and kept under normal air

C_2 : without marinating with Tom-Yum mix and kept under normal air