QUALITY CHANGES OF SARDINES (*Sardinella neopilchardus*) AT INDONESIAN AMBIENT TEMPERATURE

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ABSTRACT

In tropical countries such as Indonesia, high ambient temperature and a lack of icing cause very rapid spoilage of fish. Leaving fish uniced while waiting for processing is a common practice resulting in lower quality of the end products, and consequently lower price of the products. To study the spoilage pattern of sardines (*Sardinella neophilchardus*) in a tropical region, and to investigate a simple and quick method to judge the degree of spoilage, observations on the deterioration of sardines were carried out by incubating fish in a humidity chamber adjusted to 28-30°C, RH 70-80% for up to 14 h. The changes of sensory attributes based on demerit point score, pH, and total volatile base nitrogen (TVB-N) were monitored to determine the degree of spoilage. Although the appearance of sardines was still bright after 6-h incubation time, fish were not as fresh as those without incubation and the belly and tissue were softened. After incubation time of 10 h, fish showed slight to moderate signs of spoilage with demerit point score of 25 out of the maximum score of 39, and a TVB-N content of 20 mg%N. This is similar to the fish quality commonly used in the commercial production of dried-salted fish in Indonesia. Fish incubated for 12 h or more appeared completely spoiled and were rejected by sensory panel. A high correlation between demerit point score and TVB-N was noted; therefore the demerit point system which was more suitable, quicker, and easier could be used to determine the degree of fish spoilage.

[*Keywords*: sardines; spoilage; demerit point score; TVB-N]

INTRODUCTION

Fish is one of the most perishable flesh products and contains higher amounts of protein and water, but a lower content of connective tissue. Spoilage of fish is caused by degradation of fish tissue due to enzyme activities, biochemical changes, and microbial growth (Connell, 1980; Pedroza-Menabrito and Regenstein, 1990; Ashie *et al.*, 1996). As soon as fish die, indigenous enzymes start to actively decompose the flesh into simple products, and microorganisms originally present in gut, gills, and skin multiply rapidly. Putrefactive bacteria produce sulphur containing products with unpleasant odours and toxins (Hultin, 1991). Microbial spoilage also changes the appearance and physical properties of several fish components (Connell, 1980). Spoilage of fish proceeds differently for each fish and environmental condition.

In tropical countries such as Indonesia, high ambient temperature and a lack of icing result in very rapid spoilage of fish. Fish which can not be sold fresh are commonly preserved by traditional curing methods such as salting and drying (Yu, 1995). It is a common that raw fish which are not of prime grade are processed into either dried-salted fish or fish meal (Waterman, 1976; Connell, 1980; FAO, 1981).

Sardines are a pelagic fish found abundantly in Indonesia. In 1996, sardine production was 267,597 tons (Directorate General of Fisheries, 1998). During peak season, the production of sardines is very high resulting in an accumulation of the fish. Commonly, only good quality sardines are processed into canned fish and boiled-salted fish, while the lower grade is traditionally processed into dried-salted fish. It is a common practise to leave fish uniced while waiting for processing. This results in lower quality of the end products, and consequently decreasing the price of the products.

To determine to what extent unfresh fish are still suitable for processing into dried-salted products and to investigate a simple and quick method to judge the degree of spoilage, it is necessary to study the spoilage pattern of fish. The present paper reports the spoilage of sardines left uniced at simulated Indonesian ambient temperature.

MATERIALS AND METHODS

Sardines (*Sardinella neopilchardus*) of 25-50 g were used in this study. Fresh fish bought from the fish auction place, Sydney were transported in ice to the laboratory of UNSW, which took approximately 1 h. The fish were placed in a controlled humidity chamber, set at RH 70-80% and 28-30°C for up to 14 h

and samples (ten fish) were taken after 4 h and every 2 h afterwards. The experiment was replicated three times.

Samples were evaluated for sensory attributes, total volatile base nitrogen (TVB-N), and pH. The sensory evaluation was carried out by two trained panels on ungutted fish using the Tasmanian Food Research Unit (TFRU) scheme (Appendix 1) in which scoring (ranged from 0 to 39) was based on awarding demerit points for quality loss, i.e., higher scores indicated lower quality (Branch and Vail, 1985). TVB-N was analysed by Conway's microdiffusion method (Siang and Kim, 1992) and pH was measured using a pH meter (Orient Research Inc., Digital Ionalyzer, Model 601A) which had been calibrated with standard pH buffers of 4 and 7 (AOAC, 1990).

RESULTS AND DISCUSSION

The physical characteristics of raw materials used in this experiment are shown in Table 1. Based on sensory evaluation, the changes in quality attributes of sardines using the demerit point score system during deterioration at 28-30°C, RH 70-80% are presented in Table 2.

Table 1. Physical characteristics of fresh sardine samples.

Dimension[1]	Replicate 1	Replicate 2	Replicate 3
Length (cm)	17.0-18.5	14.0-16.0	14.5-16.5
Width (cm)	2.5-3.0	2.4-2.7	2.5-2.8
Thickness (cm)	1.5-2.0	1.3-1.8	1.5-1.7
Weight (g)	40.0-50.0	25.0-34.0	25.0-35.0

[1]Range for 10 fish

Fresh sardines bought from the fish auction place had an average demerit point score of 7, which was classified as good quality according to the classification of Branch and Vail (1985). They had a bright appearance, their skin, scale and belly were firm, and their gills had characteristics colour and smell (Table 2). After 4-h storage, the gills was slightly darker, and the belly was slightly softened and discoloured. Nasran and Arifudin (1982) also reported similar observations on Indonesian oil sardines (*S. longiceps*) incubated for 4 h at 25-26.5°C.

After 6 h, the belly started to soften and discolour; although the tissue also softened, the appearance was still bright and other attributes such as skin, scales, and vent were still in normal condition. This is

in agreement with Bremner *et al.* (1985) who noted that, in contrast to the spoilage pattern of fish stored in ice where the noticeable changes occur in the clarity and shape of eyes, the first sign of deterioration of fish stored at ambient temperature was a decrease of stiffness, while the development of mucus and odour in the gills was detected later. Gorczyca *et al.* (1985) noted that after 6-h incubation at 37°C, Australian rainbow trout (*Salmo gairdneri*) lost its sheen and the texture softened, while no off-odour was detected.

When the incubation continued to 8 h, the belly, skin, and flesh continued to lose stiffness and colour. The eyes were slightly cloudy, sunken and bloody, while the scales were slightly loose. Indonesian oil sardines showed similar changes after incubation for the same period at 25-26.5°C (Nasran and Arifudin, 1982). Sardines in the present experiment had an average demerit point score of 21, which could be categorised as fair quality. In Indonesia, fish with this quality can be sold for fresh consumption or for boiled-salted (*pindang*) products.

Spoilage was detected after 10-h incubation at which the smell of the gills and viscera were stale, the belly started to burst with moderate discoloration and the belly cavity turned to greyish. The demerit point score of sardines at this time was 25 and was classified as poor according to the classification proposed by Branch and Vail (1985). Australian rainbow trout incubated at 37°C developed off-odour after 9 h, which was evidenced as a sour odour, followed by a patrid (ammoniacal) odour after 11 h, at which time the fish was rejected in a sensory evaluation, the muscle was mushy and considerable drip was produced (Gorczyca *et al.*, 1985). Indonesian oil sardines were rejected for consumption by panellists after 10-h incubation at 25-26.5°C at which time rancid and off-odours were detected, especially that of mucus which was also sticky (Nasran and Arifudin, 1982).

The quality of sardines in the present study deteriorated after incubation times of 12-14 h, with average demerit point score of 29-34, respectively. Excessive discolouration and belly burst were noticeable, with yellow-brownish belly cavity. The appearance became dull, the skin and flesh became very soft and the vent was smelly. Sardines in such condition are not suitable for consumption. In pelagic species such as sardines, the belly is the most vulnerable part of the body, and it is the first part of the body to deteriorate (burst), especially in heavy feeding fish. This is especially due to the activity of the gut enzymes attacking the gut and the

Table 2. Changes in sardine attributes during deterioration based on demerit point scoring.

Incubation time (h)	Demerit point score (max. 39)	Changes in attributes
0	7	Appearance: bright, skin and scales firm, no slime Eyes: clear, normal in shape, no blood Gills: characteristic in colour, no mucus Belly: firm and no discolouration, cavity opalescent, blood red Vent: normal, smell neutral
4	13	Appearance: bright, skin and scales firm, slightly slimy Eyes: clear, normal in shape, no blood Gills: slightly dark in colour, fresh, no mucus Belly: soft, slight discolouration, cavity opalescent, blood red Vent: normal, smell neutral
6	16	Appearance: bright, skin and scales firm, slightly slimy Eyes: slightly cloudy, slightly sunken, slightly bloody Gills: slightly dark in colour, fishy, mucus moderate Belly: soft, slight discolouration, cavity opalescent, blood red Vent: normal, smell slightly fishy
8	21	Appearance: bright, skin and scales slightly loose, slightly slimy Eyes: slightly cloudy, slightly sunken, slightly bloody Gills: slightly dark in colour, fishy, mucus moderate Belly: soft, moderate discolouration, cavity greyish, blood dark red Vent: Slightly open, smell fishy
10	25	Appearance: slightly dull, skin soft and scales slightly loose, very slimy Eyes: slightly cloudy, sunken, very bloody Gills: slightly dark in colour, stale, mucus moderate Belly: burst, moderate discolouration, cavity greyish, blood dark red Vent: slightly open, smell fishy
12	29	Appearance: slightly dull, skin soft and scales loose, slimy Eyes: slightly cloudy, sunken, very bloody Gills: slightly dark in colour, stale, mucus moderate Belly: burst, excessive discolouration, cavity yellow-brown, blood dark red Vent: slightly open, smell fishy
14	34	Appearance: dull, skin soft and scales loose, very slimy Eyes: cloudy, sunken, very bloody Gills: very dark in colour, spoiled, mucus excessive Belly: burst, excessive discolouration, cavity yellow-brown, blood brown Vent: slightly open, smell spoiled

surrounding tissue (Connell, 1980; Huss, 1988; Bonnell, 1994). The demerit point score was highly correlated with incubation time ($r = 0.99$), and can be represented by $Y = 1.93X + 5.68$, where Y is the demerit point score and X is the incubation time in hour (Fig. 1). Bremner *et al.* (1985) assessed the sensory deterioration of four tropical fish (threadfin bream/*Nemipterus peronii*, longspine seabream/*Argyrops spinifer*, painted sweetlip/*Plectorhynchus pictus*, and snapper-like fish/*Lutjanus vittus*) at ambient temperatures (25-26°C) for up to 11 h. They found that the slope of linear correlation between demerit point score and storage time was in the range of 1.14-1.51. This indicates that sardines in the present experiment spoiled at a higher rate (1.93 demerit points h^{-1}) than the fish observed by Bremner

et al. (1985). This could be due to the different species used in the experiments, as different species behave differently during deterioration, indicating that there is a need to carefully observe the spoilage for each species.

The TVB-N level increased with increasing incubation time (Fig. 2). Before incubation, fresh sardines had an average TVB-N of 13 mg%N, which increased to 16 mg%N after 6-h incubation. When the signs of spoilage were detected (after 10-h incubation), the TVB-N level was 20 mg% N. Further incubation of sardines to 12 and 14 h in the present study increased the TVB-N levels to 23 and 31 mg%N, respectively.

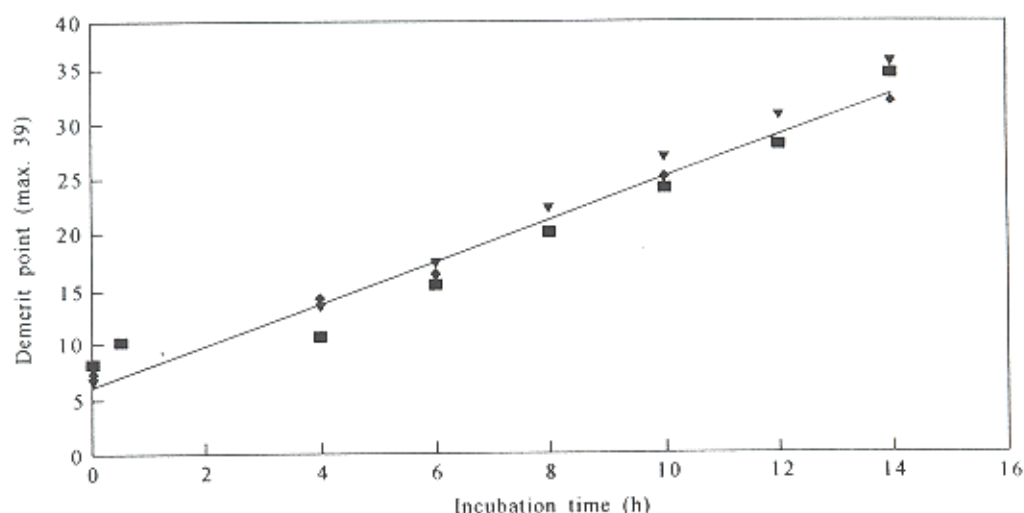The correlation between TVB-N level and incubation time was exponential and can be represented

Fig. 1. Changes of demerit point score of sardines incubated at 28-30°C (from three replicates, Y=1.93X+5.68, r = 0.99).
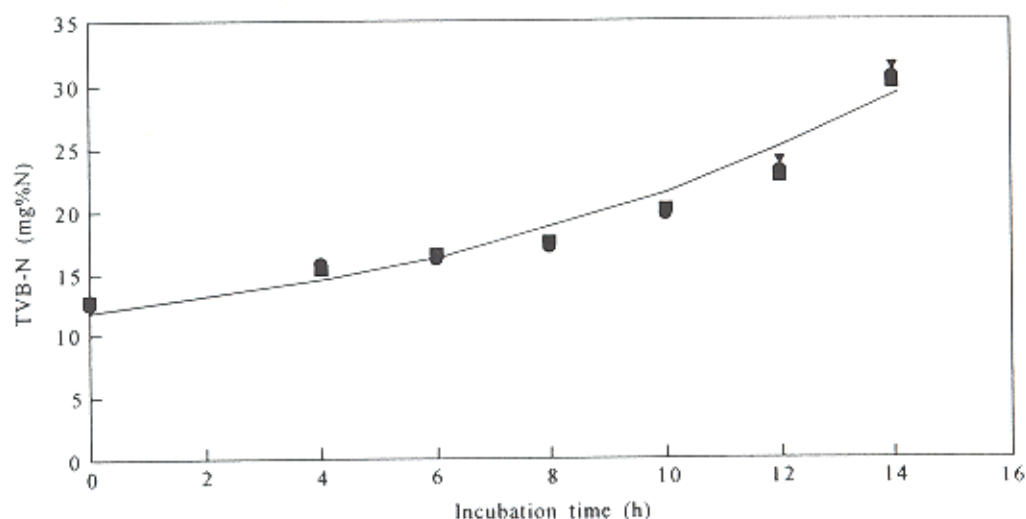

Fig. 2. Changes of total volatile base nitrogen (TVB-N) of sardines incubated at 28-30°C (from three replicates, Y=10.98e$^{0.07}$X, r = 0.97).

by Y = 10.98e$^{0.07}$X (r = 0.97), where Y is TVB-N (mg%N) and X is incubation time (h). Exponential correlation between TVB-N and incubation time was also observed by Estrada *et al.* (1985) for whiting (*Sillago maculata*) and tilapia (*Oreochromis niloticus*) stored at 29°C and RH 71%.

Generally, the limit value of TVB-N for fresh fish acceptability is around 30 mg 100 g^{-1} flesh (Sikorski *et al.*, 1990); however, this value also depends on the species of fish. The limit of acceptability of cold-water fish in ice was 30-40 mg TVB-N 100 g^{-1} flesh (Huss, 1988). For fatty fish species, such as herring and mackerel, the maximum value of TVB-N for

acceptable fish is about 20 mg 100 g^{-1} flesh (Sikorski *et al.*, 1990).

Nasran and Arifudin (1982) found that Indonesian oil sardine kept at temperature of 26-28°C was rejected by panellists (using 9 hedonic scales) after 10 h, after which time the TVB-N value reached 20 mg%N. In the present experiment, sardines showed TVB-N value of 20 mg%N after 10 h-incubation at 28-30°C and RH 78-80%, supporting the finding of Nasran and Arifudin (1982). Demersal fish, such as rabbit fish (*Siganus* sp.), were rejected after 18-h storage at temperature of 28.8-32°C, at which time the TVB-N level was 75 mg%N (Putro *et al.*, 1985). Upon incubation at 29°C

and RH 71%, whiting and tilapia had TVB-N levels of 52 mg%N after incubation of 12 h, and 15 mg%N after 16.5 h, respectively (Estrada *et al.*, 1985). At these levels, both fish were rejected by a taste panel.

Differences in the detection of spoilage or the rejection of those fish and sardines in the present study were due to species differences. The rejection time of tilapia, which was longer than that of whiting and sardines in both studies, was especially due to the lower level of TVB-N causing less offensive odour. Moreover, similar to rabbit fish, tilapia had harder and more compact skin than did whiting and sardines. The demerit point score of sardines after 10-h incubation was 25 and it can be graded as poor quality according to Branch and Vail (1985).

A linier correlation was found between TVB-N level and demerit point, and can be represented with the equation $Y = 1.48X - 8.13$ (r = 0.95), where Y is TVB-N (mg%N) and X is the demerit point score (Fig. 3). This means that the demerit point score, besides being a measure of quality deterioration of fish, can also be used to predict the TVB-N level. The demerit point score was also more reliable in predicting the remaining shelf-life of fish stored at various temperatures (Branch and Vail, 1985).

The pH of fish during incubation was relatively constant (Fig. 4). The average initial value was 6.1 which increased slightly to 6.2 after 6 h. However, the pH decreased after 10-h incubation, when spoilage was detected, and increased for the following time of
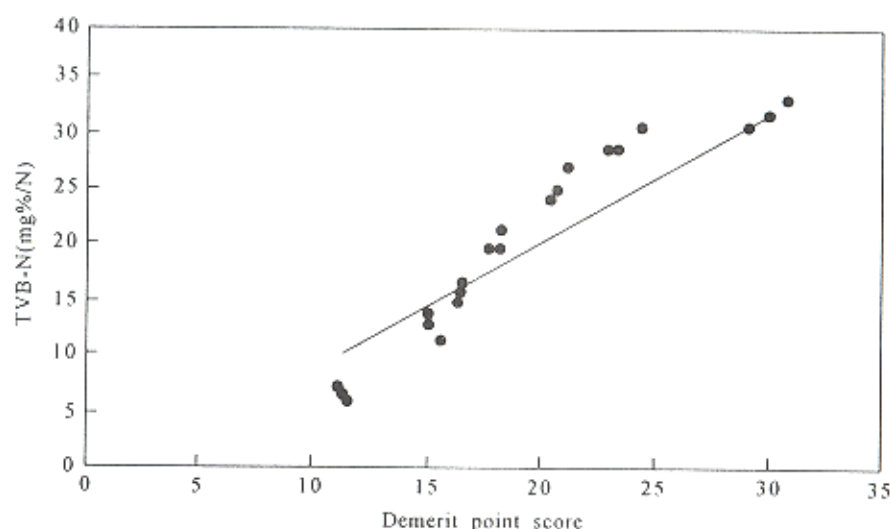

Fig. 3. Correlation between demerit point and total volatile base nitrogen (TVB-N) of sardine incubated at 28-30°C (Y= 1.48X - 8.13, r = 0.95).
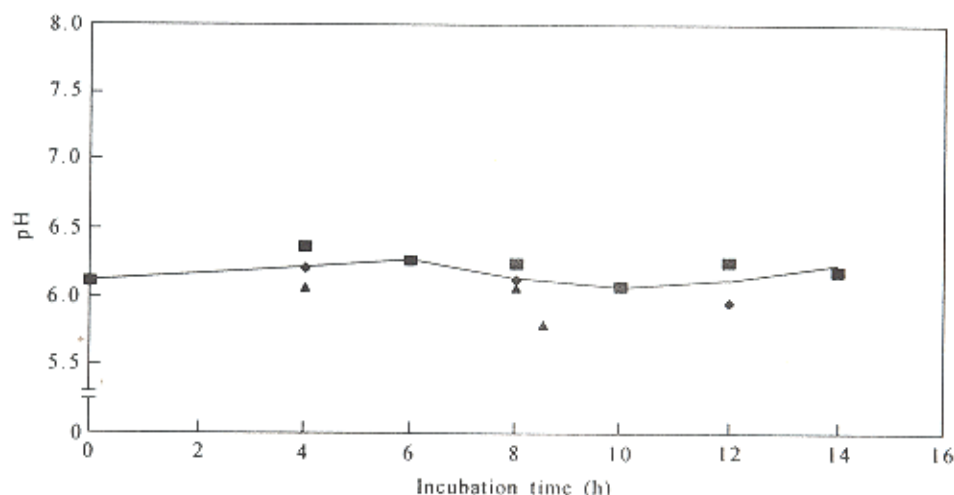

Fig. 4. Changes of pH of sardine incubated at 28-30°C (each point is the average of three replicates).

incubation but the value never exceeded 7.0. The use of pH as an index of spoilage is of no value (Krishna-kumar et al., 1986; Gelman et al., 1990).

In producing dried-salted sardines, low quality raw material is commonly used. However, this does not mean that completely spoiled sardines are suitable to be processed for dried-salted fish, since the belly of spoiled sardines is often burst and the texture of the flesh is soft and flabby. Nasran and Arifudin (1982) suggested that raw sardines suitable to be processed for drying and salting are those left at ambient temperature (26-28°C) for not more than 7 h, while the TVB-N value of raw sardines commonly used by traditional processors for dried-salted product is commonly 20 mg%N (Saleh and Murtini, 1982).

Similarly, the lowest quality sardines suitable for dried-salted fish production are preferable not completely spoiled (sardines incubated for 12 h or more), but fish that show slight to moderate signs of spoilage. This can be obtained by incubating sardines at the above conditions for not more than 10 h, which will result in a TVB-N value of approximately 20 mg%N in the flesh and a demerit point score of 25. This is in accordance with the raw sardines commonly used by traditional processor in Indonesia (Saleh and Murtini, 1982).

Utilizing the good correlation between TVB-N and demerit point which reflects the quality/freshness of sardines makes grading fish easier. Based on demerit poin scoring, sardines suitable for canning, boiled salting, dried salting or fish meal can be determined in accordance with their freshness, therefore the uniform quality of end product is assured.

CONCLUSIONS AND RECOMMENDATION

In the investigation of the spoilage pattern of sardines (S. neophilchardus) in a simulated Indo-nesian climate, it was demonstrated that pH could not be used as a spoilage indicator. Other parameters monitored, i.e., sensory characteristics (by demerit point scoring) and TVB-N (mg%N) showed a good correlation with incubation time and spoilage. Since there is a good correlation between the demerit point score and TVB-N value, either method can be used as an indicator for determining degree of fish freshness or spoilage. However, the demerit point score is considered more suitable, practical, and easier for local conditions.

. A linear correlation was found between demerit point score and incubation time, while the cor-relation of TVB-N and incubation time was found to be exponential. Since demerit point scoring is earlier and faster to measure, it can be concluded that demerit point score can be used to predict the TVB-N and hence the spoilage.

It was also concluded that, although the appear-ance of sardines was still bright after 6-h incubation time, fish were not as fresh as those without incubation (the belly and tissue softened). After incubation time of 10 h, the product showed slight to moderate signs of spoilage that was similar to those commonly used in the commercial production of dried-salted fish in Indonesia. Fish incubated for 12 h or more were completely spoiled and were rejected. For subsequent study, i.e., to evaluate the quality of dried-salted sardines derived from different raw material quality of fresh sardines, sardines incubated for 6 and 10 h will be used as raw materials.

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Appendix 1. Source sheet for sensory assesment based on TFRU scheme.

Sample code = ☐

Observation	Score
Appearance	
very bright	0
bright	1
slightly dull	2
dull	3
Skin	
firm	0
soft	1
Scales	
firm	0
slightly loose	1
loose	2
Slime	
absent	0
slightly slimy	1
slimy	2
very slimy	3
Stiffness	
prerigor	0
rigor	1
postrigor	2
Eyes	
Clarity	
clear	0
slightly cloudy	1
cloudy	2
Shape	
normal	0
slightly sunken	1
sunken	2
Irish	
visible	0
not visible	1
Blood	
no blood	0
slightly bloody	1
very bloody	2
Gills	
Colour	
characteristics	0
slightly dark, slightly faded	1
very dark, very faded	2

Observation	Score
Mucus	
absent	0
moderate	1
excessive	2
Smell	
fresh oily, metalic, seaweed	0
fishy	1
stale	2
spoilt	3
Belly	
Discoloration	
absent	0
detectable	1
moderate	2
Firmness	
firm	0
soft	1
burst	2
Vent	
Condition	
normal	0
slightly break, exudes	1
excessive, opening	2
Smell	
fresh	0
neutral	1
fishy	2
spoilt	3
Belly cavity	
Strains	
opalescent	0
greyish	1
yellow-brown	2
Blood	
red	0
dark red	1
brown	2
Marking	
very fresh = 0	
very spoiled = 39	