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QUALITY OF COD (*GADUS MORHUA*) FILLETS PACKED IN ICE AND MODIFIED ATMOSPHERES

Khairul Azam^{a*}, Claudia Ruiz-Capillas^b, William Horner^b

^aFisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh.

^bInstitute of Food Health Quality, The University of Hull, Cottingham Road, Hull, HU6 7RX, U.K.

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Abstract: The quality attributes of filleted cod (*Gadus morhua*) supplied by the industry in packages containing normal atmosphere, modified atmosphere (MAP) and in ice were compared. Fillets were stored at refrigerated temperature (0-4°C) for 15 days and sensory, biochemical and microbiological parameters were evaluated. It was clearly observed that cod fillets placed in ice were of better quality and had shelf life ranging between 11-15 days. Contrary to this result, cod fillets in MAP and normal atmosphere packages indicated poor quality and had shelf life lying between 3-7 days respectively.

Keywords: MAP; Quality; Shelf life; Iced storage; Cod fillets

Introduction

As commercial production of fish expands, manufacturers have been motivated to investigate methods for maintaining product freshness. The perishable nature of fish is well-known fact of the industry. To meet the rising demands of fresh fish in the market, prolonged shelf life of fish or increase in rejection time by different methods has become necessary. Modified Atmosphere Packaging (MAP) is a useful preservation technique for prolonging the shelf life of perishable products (Church, 1998).

Modified atmosphere packaging (MAP) involves the evacuation of air and the introduction of the gas mixture in a pack, which typically may be a tri mix of carbon dioxide (CO₂), nitrogen (N₂) and oxygen (O₂). O₂ will generally stimulate the growth of aerobic bacteria but inhibit the growth of strictly anaerobic bacteria (Farber, 1991). N₂ is used to displace oxygen in the packs so as to delay oxidative rancidity and inhibit the growth of aerobic organisms in MAP (Farber, 1991). Carbon dioxide is mainly responsible for the bacteriostatic effect on microorganisms in modified atmospheres (Farber, 1991). The bacteriostatic effect is influenced by the concentration of carbon dioxide, the load of initial bacterial population, storage temperature and type of product packaged (Reddy *et al.*, 1992). The ability of modified atmosphere packaging to extend shelf life of foods has been recognized for many years. However, it was not until 1930 that the first report on using MAP to extend shelf life of fish appeared (Killeffer, 1930). It was not until 1979 that the first MAP fish product was sold (Kimber, 1984). Many researchers now find MAP as the most favorable

* Corresponding author: Tel.: +88-(041)-724498; Fax: +88-(041)-731244; e-mail: <azamku@khulnanet.net>

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alternative for maintaining the stability of refrigerated fish for an extended period of time because fish stored under MAP is considered a fresh product. A wide variety of gases have been investigated for their potential use in MAP. (Reddy *et. al.*, 1982; Stammen *et. al.*, 1990; Gibson and Davis, 1995). The choice of gas mixture used is mainly influenced by the microbiological flora capable of growing on the product and the product sensitivity to O₂ and CO₂. However, very high concentrations of CO₂ are reported to produce sensory changes (Haard, 1992).

The present investigation was carried out in order to assess the quality of cod fillets from the industry packed in ice, in normal atmosphere and modified atmospheres and stored at refrigerated temperature (0-4⁰ C).

Materials and Methods

Seachill Ltd. (an industry supplying fresh fish all over the UK) delivered Cod (*Gadus morhua*) fillets packed in commercial polystyrene boxes to the laboratory at the Institute of Food Health Quality (FHQ), The University of Hull. There were three boxes marked as sample A, sample B and sample C. Sample A had been packed with modified atmospheres (75:24:1; CO₂: N₂: O₂), the present practice of preservation used by the industry. Fillets in sample B were packed in polythene pouches having normal atmosphere. While sample C had fillets that were covered with complete ice (1:1, ice: fish). The boxes were stored in a refrigerator set at 4-6⁰ C for a period of 15 days. At set intervals (0, 3, 7, 9, 11 and 15 days), two fillets contained in one pouch from each boxes of Sample A and Sample B and two fillets from Sample C were removed. One fillet was used for biochemical and microbiological analyses while the other fillet was used for sensory evaluation.

Sensory Assessment: Visual inspection was carried out according to the scheme of Botta (1995) with slight modification (Table 1.)

Table 1. Visual Inspection of raw cod fillet.

Factors Assessed	Observed Characteristics	Demerit Point
Appearance	Translucent	0
	Slightly discolored	1
	Slightly opaque	2
	Opaque	3
Skin color	Very bright	0
	Bright	1
	Slightly dull	2
	Dull	3
Texture	Firm	0
	Slightly soft	1
	Soft	2
Odor	Fresh marine	0
	Odorless or slightly musty,	1
	Ammonical, Sour	2
	Very ammonical, very sour	3

For the preparation of cooked samples, fillets were steamed for 10-15 minutes in a microwave oven and served hot. The scores of 5-6 judges drawn from the members of the laboratory were averaged to give one mean observation per storage time. Scores were given on the basis of the scheme shown in Table 2.

Table 2. Quality assessment of cooked cod fillets (Taste panel).

Quality Parameter	Characteristics	Score	Sample A	Sample B	Sample C
Odor	Sweet, marine and seaweedy	0			
	Loss of odor	1			
	Neutral	2			
	Slightly cold storage odor (cardboard), slightly citric, amine	3			
	Cold storage odor (cardboard), citric, strong amine	4			
Color	White and opalescent	1			
	Loss of whiteness	2			
	Greyish, one small blood stain	3			
	Slightly yellow, a few small blood stains	3			
	Light brown, discolored of blood	4			
Flavor	Sweet, marine and seaweedy	0			
	Loss of taste, slightly sweet	1			
	Neutral	2			
	Slightly cold storage flavor (cardboard), insipid, slightly soap, slightly citric, slightly amine	3			
	Cold storage flavor (cardboard), dry fish soap, citric, strong amine	4			
	Very succulent, flaky and coherent also succulent after chewing several times	0			
	Succulent, flaky and coherent. Succulent after chewing several times	1			
Texture	Initial feeling is succulent, watery after first chewing. Dry and tough during following chewing	2			
	Dry, fibrous and/or tough. Dry and tough from the first chewing	3			
	Very dry, fibrous and/or tough. Very tough from the first chewing	4			

Chemical Analyses

Determination of TMAO-N, TMA-N and TVB-N

Preparation of samples: For determinations of TMA-N and TMAO-N, 50g minced fish was blended with 100ml of 7.5% TCA and filtered through Whatman N° 1 paper while for TVB-N, 10g of minced fish was blended with 90ml of 7.5% TCA and filtered.

Preparation of standard: Standards of ammonium chloride (NH₄Cl) (in the case of TVB-N determination) or trimethylamine hydrochloride (TMA-HCl) (in the case of TMA-N determination) in the range of 50-500 μmole/L and 50-200μmole/L respectively were prepared. 100μl of these standards were injected into the FIA manifold that was then closed.

Flow Injection Analysis for Determination of TMAO-N, TMA-N and TVB-N: The determination of TVB-N and TMA-N using the FIA technique was performed according to the methodology used by Sadok *et al.*, (1995) which had been slightly modified. The modification was an increase of concentration of NaOH of 0.6M to 1M, and an increase in concentration of formaldehyde from 12% to 20%. TMAO-N was determined by reducing TMAO-N to TMA with 15% TiCl₃ at 100°C

until the color disappeared. The concentration were then be found using the FIA system. Muscle pH was measured on a 2:1 (water: fish) homogenate using a glass electrode at ambient temperature.

Microbiological Analyses

From each fillet, a sample of 10g was diluted ten fold (90ml) in peptone water and homogenized for 30s in a stomacher. The homogenates were further diluted in peptone water. In all experiments, counts of total viable organisms, lactic acid bacteria (LAB), H₂S producing bacteria *Photobacterium phosphoreum* and were enumerated as pour plate counts in nutrient agar, MRS agar and iron agar containing 0.5% sodium chloride respectively. Nutrient agar plates were incubated at 25°C for 72 hours while MRS agar plates at 25°C for 7 days. Plates for the growth of *Photobacterium phosphoreum* were incubated at 15°C for 7 days. Iron agar plates were incubated at 25° C for 72 hours.

Results and Discussion

Sensory Assessment

Raw fillet: Changes in the attributes of the raw filets during chill storage were compiled using the descriptions provided in Table 1. Changes occurring in the surface appearance, odor, texture and skin color of the fillets were found to be useful in assessing freshness quality and appeared to have potential for use in the routine evaluation of cod fillets.

Odor of the fillet for sample A was quite intense (stale) on the 3rd day of storage (Table-3) which decreased (on the 7th day) and then increased progressively with storage time. Odor in the fillet for sample B rose sharply on the 7th day of storage and found to be spoiled on the 9th day. However, odor in sample C remained normal until day 7 and produced only fishy odor on the 11 day of storage. Even at the end of storage period, odor of sample C were found to be only stale and not spoiled. Considering the limit of rejection for odor, it was found that sample A and sample B lost their acceptability by day 9 while rejection time for sample C fillet remained between 11 and 15 days of storage.

Texture score was in agreement with that of odor score. Texture was found to be completely soft for sample A and sample B fillets from day 9 onward while fillets in sample C were found to be firm until 9th day of storage. Texture score indicated limit of rejection time for sample A and sample B between 7 and 9 days and for sample C between 11 and 15 days respectively.

Color of the fillets did not vary significantly amongst the three samples. Rejection time for all the three samples remained between 9 and 11 days. A score of 2 for sample B on the 7th day of storage indicated a variation between fish to fish and fillet to fillet.

Skin color was found to be very dull for sample A from 3rd day of storage onwards while score of skin color for sample B increased progressively with storage time. In sample C, skin color remained bright until day 9 and was slightly dull at the end of storage trial. Rejection time for sample A and sample B initiated from day 3 and day 9 while rejection limit for sample C fillet remained between 9 and 11 days.

Table 3. Sensory score (Visual Inspection) of cod fillets.

Factor Assessed	Sample	Storage Days					
		0	3	7	9	11	15
Appearance	A	0	0	0	1	2	3
	B	0	0	2	1	3	3
	C	0	0	1	1	2	2
Skin Color	A	0	3	3	3	3	3
	B	0	1	1	2	3	3
	C	0	0	0	1	2	2
Texture	A	0	2	1	2	2	2
	B	0	0	1	2	2	2
	C	0	0	0	1	1	1
Odor	A	0	2	1	3	2	3
	B	0	0	2	3	3	3
	C	0	0	0	1	1	2

Cooked fillet: Demerit points for flavor in cooked fillet varied between 0 and 4 and the limit of rejection was 2.0. For sample A and sample B fillets, this limit was found to lie between 3 and 7 days. From day 7 onwards, a strong amine flavor was perceived. However, it was clearly observed that sample C had comparatively better flavor and the shelf life remained between 9 and 11 days. Only strong amine flavor was perceived at the end of storage period. (Table 4).

Table 4. Changes in flavor of cod fillets (cooked) as judged by taste panel.

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	0.58	1.67	3.20	3.60	3.67	3.63	2.00	3-7
B	0.58	0.67	3.60	3.80	4.00	4.00	2.00	3-7
C	0.58	1.00	1.80	2.00	2.33	3.38	2.00	9-11

Changes in the score of texture for cooked fillets are presented in Table 5. Shelf-life regarding texture for sample A and sample B remained within 3-7 days while for sample C between 7-9 days respectively. However, reduced shelf life with regard to texture in cooked fillet particularly for sample C may be attributed to score provided by some inexperienced taste panel members. From 7th day, cooked fillet was found to be succulent and tough. This score of texture did not concur with the results of other sensory attributes of cooked fillets.

Table 5. Changes in texture of cod fillets (cooked) as judged by taste panel.

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	1.00	1.00	2.40	3.20	3.33	3.75	2.00	3-7
B	1.00	0.67	2.80	3.40	3.67	3.75	2.00	3-7
C	1.00	0.83	2.00	1.75	3.00	3.25	2.00	9-11

Table 6 summarizes the score of odor of cooked fillets as judged by the taste panelists. With rejection limit of score 2.0, shelf-life of sample A and sample B were within 3-7 days while for sample C, shelf-life varied between 11-15 days. This result was in agreement with other measurements. Odor (amine) increased abruptly from 7th day for sample A and sample B. In the case of sample C, strong odor of amine was only perceived at the end of storage period.

Table 6. Changes in odor of cod fillets (cooked) as judged by taste panel.

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	0.83	1.67	3.80	3.60	3.33	4.00	2.00	3-7
B	0.83	1.33	3.60	4.00	4.00	4.00	2.00	3-7
C	0.83	1.33	2.00	2.20	2.00	3.25	2.00	11-15

Results of color of cooked fillet for different samples are provided in Table 7. Slight loss of whiteness was observed in sample C until 11 days of storage., sample A and sample B indicated loss of whiteness from day 3 and crossed the limit of rejection by the day 7.

Table 7. Changes in color of cod fillets (cooked) as judged by taste panel.

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	0.50	1.00	2.80	2.80	2.33	4.00	2.00	3-7
B	0.50	0.67	2.60	3.40	3.67	3.75	2.00	3-7
C	0.50	0.67	1.40	1.40	1.67	3.00	2.00	11-15

According to overall acceptability (odor, color, flavor and texture) score, sample A and sample B indicated shelf life between 3-7 days and sample C between 11-15 days respectively. (Table 8). Thus it was clearly observed that sensory score of cooked fillet showed relatively better quality of product for sample C in comparison to sample A and sample B.

Table 8. Overall acceptability of cod fillets (cooked) as judged by taste panel.

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	0.73	1.34	3.05	3.30	3.17	3.85	2.00	3-7
B	0.73	0.84	3.15	3.65	3.84	3.88	2.00	3-7
C	0.73	1.04	1.90	1.85	2.08	3.22	2.00	11-15

Biochemical Analyses

Changes in the concentration of TMA-N for samples A, B and C are summarized in Table 9. Very low concentration (0.03 mg/100g) of TMA-N was observed on the 0th day. The concentration remained comparatively low for sample C until 11 days (8.06 mg/100g). However, in the case of sample A and sample B, concentration of TMA-N increased abruptly on the 7th day (39.43 and 33.63 mg/100g) and then increased slowly indicating almost double the value of sample C at the

end of storage period. In their cases (sample A and sample B), shelf life remained only between 3-7 days (having rejection limit of 12-15 mg/100g).

Table 9. Changes in the concentration of TMA-N (mg/100g) in cod fillets

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	0.03	6.83	39.43	43.69	40.79	42.24	12-15	3-7
B	0.03	1.17	33.63	39.34	41.19	42.24	12-15	3-7
C	0.03	0.08	1.19	2.72	8.06	21.12	12-15	11-15

Concentration of TVB-N followed a similar pattern to that of TMA-N. (Table 10) However, the initial (day 0) value of TVB-N was very high (10.95 mg/100g). For sample A and sample B, the value of TVB-N exceeded the limit of rejection after the 3rd day. But for sample C, the limit was crossed after 11 days of storage. At the end of storage period, TVB-N value for sample B was found to be very high (135.85 mg/100g).

Table 10. Changes in the concentration of TVB-N (mg/100g) in cod fillets.

Sample	Days of Storage						Acceptability Limit	Shelf-Life
	0	3	7	9	11	15		
A	10.95	20.13	61.40	69.50	74.63	82.63	25-30	3-7
B	10.95	13.36	51.48	77.63	97.65	135.85	25-30	3-7
C	0.03	0.08	1.19	2.72	8.06	21.12	12-15	11-15

During the trial period, appreciable pH change for sample A and sample B were observed. pH showed higher value (>7.30) from the 7th day for sample A and sample B. In the case of sample C, the pH was slightly acidic (pH 6.98) until day 11 and then increased slightly (pH 7.20) at the end of the investigation (Table 11).

Table 11. Changes in pH in cod fillets.

Sample	Days of Storage					
	0	3	7	9	11	15
A	6.80	7.01	7.35	7.12	7.29	7.30
B	6.80	6.81	7.59	7.41	7.70	7.71
C	6.80	6.80	6.89	7.01	6.98	7.20

Microbiological Analyses

Table 12 shows total viable counts (TVC) for different samples. TVC increased by almost one log cycle for sample C from the 0th day until the 11 day but then increased unto two log cycles on the 15th day (log 8.72). Sample B indicated a sharp increase of almost 3 log cycles from log 6.25 on the 7th day to log 9.18 on the 9th day of storage. TVC in sample A was found to be lying between counts in sample B and sample C. Shelf-life related to TVC remained between 3-7 day (limit of rejection of log 6.00) for sample A and sample B. While shelf life of cod fillet for sample C lay between 11 and 15 days.

Table 12.Total Viable Counts in cod fillets (log number)

Sample	Days of Storage						Acceptability	Shelf-Life
	0	3	7	9	11	15		
A	4.71	5.02	6.40	7.01	7.72	8.95	6.00	3-7
B	4.71	4.56	6.25	9.18	9.74	10.46	6.00	3-7
C	4.71	4.69	5.53	6.08	6.88	8.72	6.00	11-15

Counts of *Photobacterium phosphoreum* showed higher values for sample A and sample B in comparison to sample C (Table 13). In these samples (A and B) large number of *Photobacterium phosphoreum* (log 7.42) was observed from the 7th day, which increased to log 8.34 and log 9.79 at the end of 15 days

Table 13.Counts of *Photobacterium phosphoreum* in cod fillets (log numbers)

Sample	Days of storage					
	0	3	7	9	11	15
A	4.95	5.16	6.06	6.96	7.83	8.34
B	4.95	4.72	7.42	7.58	8.96	9.79
C	4.95	5.10	6.17	6.05	7.19	7.97

Growth of lactic acid bacteria (LAB) was found to be extremely low (<30 colonies) for sample C throughout the study period as expected. (Sample C had fillets in aerobic environment). Also on the initial day (day 0), no count was done on any sample because the numbers were found to be lower than 30. However, interestingly, LAB was observed in sample A and sample B from day 3 and day 7 onward and increased progressively with storage time indicating an anaerobic environment in the pouches. Probably, the pouches were filled with modified atmosphere.

Table 14. Lactic acid bacteria in cod fillets (log numbers)

Sample	Days of storage					
	0	3	7	9	11	15
A	*	3.81	4.86	5.63	6.63	7.39
B	*	*	5.88	6.89	7.51	7.81
C	*	*	*	*	*	*

* no colonies were observed

MAP packaging had in general an inhibitory effect on the growth of microflora (aerobic and anaerobic bacteria, H₂S producing bacteria and Enterobacteriaceae) but limited inhibition on the production of TVB and TMA (Devere and Boskou, 1996). The present study indicated a similar pattern of result with regard to production of TVB and TMA. However, H₂S producing bacteria increased significantly even in the MAP pack. Spoilage of fish stored at refrigerated temperature is mainly due to *Shewanella species* which are strongly inhibited by the presence of increased concentration of carbon dioxide in MAP packs (Boskou and Devere, 1997). However, *Photobacterium phosphoreum* has been highly resistant to carbon dioxide (Huss, 1995) and it has an increase growth rate under anaerobic conditions. It is these factors that explain the importance of *Photobacterium phosphoreum* in the spoilage and reduced extension time of shelf life of fish stored in MAP packs (Davies, 1997). The results of the present investigation showed that carbon dioxide did not inhibit the growth of *Photobacterium phosphoreum* in complete agreement to

results obtained by Davies (1997). Contrary to these results, extension of shelf life in MAP has been attributed to the effect of CO₂ inhibiting the growth of specific spoilage organisms particularly *Photobacterium phosphoreum* (Dalgaard, 1998).

MAP has been primarily used for the extension of shelf life. However, the reported increases in shelf life for fish vary markedly and are only small in comparison with those reported for other products. Reddy *et.al.*, (1982) observed that the percentage increase in shelf life of fish stored in MAP ranged from 0% (no increase) to 280%. The present investigation did not show any indication of the extension of shelf life of cod fillets using MAP. One of the other important factors in MAP technology is the gas-product ratio (Church, 1998). It may be attributed that the gas product ratio were not appropriate in the MAP packets supplied. The beneficial effect of ice has been clearly observed.

Conclusion

The results clearly indicated that cod fillets placed in ice (sample C) were better quality with respect to sensory, biochemical and microbiological parameters compared to sample A (modified atmosphere) and sample B (normal atmosphere). Further investigation is needed to establish and precisely justify that MAP technology has beneficial effects under the circumstances stated.

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