QUALITY ASSESSMENT OF BLUE WHITING (*Micromesistius poutassou*) DURING CHILLED STORAGE BY MONITORING LIPID DAMAGES

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ABSTRACT

Different kinds of lipid damage indices (peroxide value, conjugated diene index, thiobarbituric acid index, free fatty acids content, polyene index and fluorescent compounds formation) were studied during the chilled storage (0ºC) of a lean fish species (blue whiting, *Micromesistius poutassou*) and compared to total volatile base-nitrogen content (TVB-N). Similar to previous results regarding a fatty fish species (sardine), fluorescence detection of interaction compounds calculated as the ratio between fluorescence measured at 393/463 nm and 327/415 nm showed the best correlation with TVB-N evolution and provided the highest independent contribution to time prediction during chilled storage. Present results indicate that this fluorescence detection is sensitive enough for assessing freshness loss during chilling of a lean fish species and appears to be the equal if not the superior of a recognized method such as TVB-N in order to assess fish spoilage.

Running Head: Quality assessment and lipid damages

Key Words: Blue whiting, chilling, fluorescence, interaction compounds, lipid damage, quality

INTRODUCTION

During processing and storage, fish quality may decline as a result of several factors. One of the most important concerns the oxidation of highly unsaturated lipids directly related to the production of off-flavors and odors in foods (Pearson et al., 1977; Pigott and Tucker, 1987). Many methods have been used to measure primary (peroxides) and secondary (carbonyl compounds) oxidation products in foods for quality assessment (Melton, 1983; Kim and Labella, 1987). In addition, detection of interaction compounds formed by reaction of oxidation products and biological amino constituents (proteins, peptides, free amino acids and phospholipids) has also been employed for quality changes determination (Maruf et al., 1990; Lubis and Buckle, 1990; Aubourg and Medina, 1997).

Research on quality changes during chilling of lean fish species has been focused on the non-lipid effects concerning changes in sensory attributes, formation of volatile amines and hypoxanthine and changes in proteins and physical properties of the muscle (Smith et al., 1980; Chalmers et al., 1992; Olafsdóttir et al., 1997). However, most of these methods have only been used in research and sensory analysis is most often used to assess the freshness of fish in the industry.

Very few studies have investigated the lipid changes during a lean fish species chilling. It has been proven that free fatty acids formation could be an accurate freshness index during hake (*Merluccius hubbsi*) chilling (Barassi et al., 1987). In addition, hydrolysis has been reported to influence the formation of oxidation products and affect fish quality (Miyashita and Takagi, 1986).

In the present work, the lipid damage during chilled storage of a lean fish species (blue whiting) was studied to propose a quality method for testing fish stability. Different lipid indices were checked and compared to a commonly employed method (total volatile base-nitrogen content) to assess fish spoilage during chilling. A great attention was focused on the fluorescence detection of the interaction compounds resulting from reaction between oxidized lipids and biological constituents (Gardner, 1979; Leake and Karel, 1985), based on previous experiences on fatty fish species (Aubourg and Medina, 1997; Aubourg et al., 1997, 1998).

MATERIALS AND METHODS

Raw material, processing and sampling: Fresh blue whiting (*Micromesistius poutassou*) were obtained 10 hr after catching; during this time the fish had been kept on ice. Upon arrival in our laboratory, whole individual fish were stored (on ice) in isothermal rooms at 0°C. Blue whiting was divided into three batches. Sampling was undertaken at day 0, 2, 6, 10, 13 and 15. For it, in each batch analyses were carried out on the homogenized white muscle of the whole fillet from three individuals.

Basic analyses and total volatile base-nitrogen (TVB-N) determination*:* Water content was determined by weight difference between the fresh homogenized muscle (1-2 g) and after 24 hr at 105 °C. Results are expressed as g water/100 g muscle. Lipids were extracted by the Bligh and Dyer (1959) method. Quantification results are expressed as g lipids/100 g wet muscle.

TVB-N were measured by the Antonacopoulos (1960) method with some modifications. Ten grams fish muscle were extracted with perchloric acid (6 %) and made up to 50 mL. TVB-N content was obtained by steam distillation of the acid extracts made alkaline to pH 13 with NaOH (20 %), followed by titration of the distillate with 10 mM hydrochloric acid. Data are expressed as mg TVB-N/100 g muscle.

Lipid damage measurements: Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids.

Peroxide value (PV) expressed as meq oxygen/Kg lipids was determined by the ferric thiocyanate method (Chapman and McKay, 1949).

Conjugated diene (CD) formation was measured at 233 nm (Kim and Labella, 1987). The result is expressed according to the formula: CD = B x V / w, where B is the absorbance reading at 233 nm, V denotes the volume (mL) of the sample and w is the mass (mg) of the lipid extract measured.

The thiobarbituric acid index (TBA-i) (mg malondialdehyde/kg sample) was determined according to Vyncke (1970).

Fatty acid composition*:* Lipid extracts were converted into fatty acid methyl esters by the Lepage and Roy method (1986) and analyzed by Gas Chromatography according to Medina et al. (1994). The polyene index (PI) was calculated as the following fatty acids ratio: 20:5 + 22:6 / 16:0 (Lubis and Buckle, 1990).

Fluorescence analyses: Fluorescence formation (Perkin-Elmer LS 3B) at 393/463 nm and 327/415 nm was studied as described earlier (Aubourg and Medina, 1997; Aubourg et al., 1997, 1998). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the sample fluorescence at each excitation/emission maximum, and F_{st} is the corresponding fluorescence intensity of a quinine sulfate solution (1 μg/mL in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence shift (δF) was calculated as the ratio between both RF values: $\delta F = RF_{393/463nm} / RF_{327/415nm}$, and was analyzed on the aqueous (δF_{aq}) and organic (δF_{or}) phases resulting from the lipid extraction (Bligh and Dyer, 1959).

Statistical analyses: Data from the different quality measurements were subjected to the ANOVA one-way method according to Sokal and Rohlf (1981). Comparisons of means after ANOVA test were performed using a least squares difference (LSD) method (Statsoft, 1994). Multiple regression were calculated by Forward Stepwise Regression using the Statistica package (Statsoft, 1994). Non linear estimation models were calculated by using Quasi-Newton and Symplex methods (Statsoft, 1994).

RESULTS AND DISCUSSION

Water contents ranged between 80% and 85% in all samples; a slight increase during the storage was observed that could be explained as a result of contact with ice (Aubourg et al., 1997). Lipid contents ranged between 0.50% and 0.75%; no significant differences ($p<0.05$) were obtained as a result of the chilling storage.

Quality measurements (Table 1)

TVB-N content did not differ during the first 10 days of storage, showing that such index is not a good freshness degree indicator (Oehlenschläger, 1997). Then, increases were observed after 13 and 15 days, indicating the end of the lag phase of microorganisms (Whittle et al., 1990). The amount of volatile amines has been widely employed for the estimation of fish quality during the chilling process. Present results agree with previous research on fatty fish (Bennour et al., 1991; Nunes et al., 1992) and lean fish (Smith et al., 1980; Chalmers et al., 1992) where a sharp increase on volatile amines content begins after 9-10 days of storage.

A slight increase was obtained in the hydrolysis development (FFA content) during the whole storage. However, significant differences were only obtained after 13 and 15 days. The lipolytic activity of fish during chilling has shown to be species and tissue site dependent (Whittle et al., 1990); however, Barassi et al. (1987) found this detection useful in order to assess quality in hake. Previous work on sardine (Nunes et al., 1992) showed an increase after 12 and 14 days of storage, which is in accordance with present results.

On the basis of primary oxidation products (PV), fish muscle oxidized with a 10-13 days induction period. After that time, a significant increase was observed. During chilled storage of sardine (Nunes et al., 1992) higher values on PV were obtained, that could be explained as a result of being a fatty fish species.

Conjugated dienes formation showed very little significant differences. It is concluded that this method was not suitable as a quality measurement since dienes are relatively unstable and capable of interacting with other constituents (Shimasaki et al., 1977; Cho et al., 1989).

Secondary lipid oxidation products were measured by the TBA-i. An increase was observed after day 6 but then little differences were observed. This method was not found sensitive enough to provide great differences during the storage, since relatively low values were obtained by comparing them with other related experiences concerning fatty fish species (Nunes et al., 1992; Aubourg et al., 1997). It has been argued that the formaldehyde produced during the storage of formaldehyde-forming fish species could interfere the thiobarbituric acid test, so that lower TBA-i could be obtained (Careche and Tejada, 1988).

No significant differences were obtained during the first days of storage for the polyene index; decreasing values were found after 13 and 16 days, as a result of the PUFA damage. During a drying-salting process, the polyene index showed a significant ($p<0.01$) correlation with rancidity score (Lubis and Buckle, 1990). However, this method has been reported to be not sensitive enough to measure lipid damages during the first steps of lipid oxidation (Labuza, 1971).

The fluorescence ratio (δF) between two excitation/emission maxima (393/463 nm and 327/415 nm) was studied in the same way as described earlier for fatty fish species (Aubourg and Medina, 1997; Aubourg et al., 1997, 1998). Previous experiments have shown that as a result of the lipid damage increase during storage and processing, fluorescent compounds formed in the first stages led to the formation of other fluorescent compounds showing excitation/emission maxima at higher wavelengths than their precursors.

In the present study, the δF value was studied in the organic (δF_{or}) and aqueous (δF_{aq}) phases resulting from the Bligh and Dyer (1959) lipid extraction. Measurements in the organic phase showed an increase after 2 days and a decrease after 10 days. Then, no more variations were observed. This is contradictory to previous results which showed that δF_{or} correlated well with storage time and other valid quality indices used for chilled (Aubourg et al., 1997) and frozen

(Aubourg et al., 1998) fatty fish species. In addition, fluorescent compounds studies of fatty fish species carried out on organic extracts (lipids) showed high correlation with sensory measurements and storage time (Maruf et al., 1990; Lubis and Buckle, 1990).

On the other hand measurements in the aqueous phase (δF_{aq}) showed a progressive increase throughout the whole experiment, specially after 13 and 15 days, which is in agreement with previous work on sardines (Aubourg et al., 1997, 1998).

The relative formation of fluorescent compounds that are lipid- and water-soluble was studied by means of the $\delta F_{or} / \delta F_{aq}$ ratio. During the first two days of chilled storage a significant increase in this ratio was detected; then a gradual decrease was observed till the end of the storage since the δF_{aq} value was increasing. This result agrees with previous research on fatty fish species (Aubourg et al., 1997, 1998) where as long as lipid damage increases, fluorescence detected in the aqueous phase predominates; however, lower values were now obtained. Fluorescent substances formed from oxidized membrane lipids have been reported to remain attached to the amino constituent resulting in compounds quite insoluble in organic solvents (Shimasaki et al., 1984; Iio and Yoden, 1988).

Relationship between quality measurements and chilling storage

Most changes in food constituent can be related to quality loss. The changes that are continuous during processing could be employed for assessing the quality state of a product. A search for accurate indicators to measure quality degree of fish was undertaken, based on the quality indices studied.

All variables measured were subjected to multivariate regression analysis to test redundancy and to define significance for predicting time of chilling storage. Results showed δF_{aq} and TBA-i as the most useful parameters to establish a linear dependence with time ($R^2 = 0.88$; Table 2), δF_{aq} having the highest independent contribution to time prediction (Beta = 0.7657). The remaining variables were redundant to explain time, therefore they were excluded from the model. TVB-N, a

recognized parameter to estimate chilled fish quality loss was found highly correlated to δF_{aq} (R^2 = 0.92), which explains its lack of significance on the multivariate regression. δF_{aq} also showed a marked positive correlation with the extent of lipid hydrolysis occurred (R^2 = 0.83). That effect is likely related to a higher susceptibility of free fatty acids to suffer oxidation than esterified lipids (Cheftel and Cheftel, 1976). Free fatty acids have been also described to induce negative changes on the original protein structures (Careche and Tejada, 1994). There was also an important negative correlation between PV and CD (R^2 = - 0.82) reflecting decomposition of PV to give conjugated secondary oxidation products.

Optimal univariate linear and non linear regressions between each index and both chilled storage time and a recognized quality method such as TVB-N were established. Results shown in Table 3 demonstrate a higher non linear dependence of δF_{aq} on storage time than all the other variables (R^2 = 0.94). δF_{aq} followed an exponential increase with time of chilling and their evolution was closer to TVB-N than all the rest of parameters measured (R^2 = 0.86; Figs. 1 and 2).

Thus, the two variables that resulted statistically significant to time prediction (δF_{aq} and TBA-i), were subjected to non linear bivariate estimations in order to calculate regression parameters (Table 4A). By using Quasi-Newton and Symplex methods, a significant additive non linear regression for time prediction was obtained explaining a 94% of the total variance data, which is an evaluating measurement of the model fitting. That proportion of variance accounted for the independent variable is equivalent to the R-square, coefficient of determination. Parameter λ related to TBA-i contribution did not result significant for the regression, so the appropriateness of this overall model is the same as univariate logarithmic regression for δF_{aq} (R^2 = 0.94; Table 3). TBA-i was not significant for TVB-N prediction either (Table 4B). The non linear estimation model allowed to explain a 86% of the total variance data that means the same coefficient of determination (R^2) calculated for univariate non linear regressions (Table 3). Other non linear models with different relationship between both variables (δF_{aq} and TBA-i) showed the same results.

δF_{aq} has arisen as the most important contributing variable to time prediction during chilled storage of a lean fish species and for the monitoring of quality changes. Its exponential increase during storage was highly correlated to that of TVB-N. Linear and non linear estimation models calculated to predict the storage time have shown higher determination coefficients for δF_{aq} than for TVB-N; measurements of fluorescence into the aqueous phase should be considered as a more practical tool to follow changes in fish quality. On the basis of δF_{aq}, stability of a lean fish species during chilled storage has shown two days induction period and then, significant degradation takes place. Since values of TVB-N were not significantly different until day 13, δF_{aq} has also shown to be a more sensitive method to measure freshness loss. On these basis, measurement of δF_{aq} appears as a highly sensitive, rapid and accurate method to be applied on fish technology.

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FIGURE LEGENDS

Fig. 1. Non linear regression between time of chilled storage and δF_{aq}.

Fig. 2. Linear relationship between δF_{aq} and TVB-N.

Table 1. Measurements* of lipid degradation and TVB-N formation during chilling storage. Significance was declared at P<0.05.

TIME (days)	TVB-N	PV	CD	TBA-i	FFA	PI	δF_{or}	δF_{aq}	$\delta F_{or}/\delta F_{aq}$
0	17.84±1.03^{a}	2.80±0.15^{a}	5.80±1.16^{b}	0.00±0.00^{a}	13.00±6.48^{a}	3.72±0.10^{b}	0.74±0.53^{a}	8.25±3.54^{a}	0.09±0.05^{a}
2	19.67±2.84^{a}	1.93±0.77^{a}	6.85±0.45^{b}	0.03±0.02^{a}	18.60±0.57^{ab}	3.76±0.19^{b}	2.99±1.14^{b}	10.13±1.81^{ab}	0.31±0.17^{b}
6	22.74±1.63^{a}	2.40±0.63^{a}	6.25±0.34^{b}	0.42±0.04^{b}	19.22±2.37$^{a\,b}$	3.64±0.11^{b}	2.11±0.91$^{a\,b}$	21.35±4.41^{bc}	0.10±0.02^{a}
10	21.01±1.10^{a}	7.67±4.35^{ab}	5.24±0.76$^{a\,b}$	0.28±0.07^{b}	16.78±2.14^{ab}	3.77±0.09^{b}	0.95±0.30^{a}	29.82±8.73^{c}	0.03±0.01^{a}
13	32.46±3.62^{b}	15.76±7.50^{b}	4.89±2.23$^{a\,b}$	0.40±0.11^{b}	20.52±3.65^{bc}	3.58±0.33^{a}	1.25±1.12^{a}	84.35±10.94^{d}	0.01±0.01^{a}
15	37.74±4.74^{c}	17.07±4.92^{b}	3.78±0.64^{a}	0.57±0.16^{b}	31.42±3,40^{d}	3.58±0.06^{a}	1.12±0.10^{a}	143.33±9.20^{e}	0.01±0.00^{a}

* Mean values of three determinations ± standard deviation. Values in the same column followed by different letters are significantly (p<0.05) different.

** Abbreviations: TVB-N (total volatile base-nitrogen), PV (peroxide value), CD (conjugated dienes), TBA-i (thiobarbituric acid index), FFA (free fatty acids), PI (polyene index), δF_{aq} (fluorescence shift ratio of the aqueous phase), δF_{or} (fluorescence shift ratio of the organic phase)**.**

Table 2. Regression summary for time of chilled storage (coefficient of multiple correlation: R^2= 0.88). BETA: standardized regression coefficient. Significance was declared at $P < 0.05$. Abbreviations as specified in Table 1.

	BETA	**Standard Error of BETA**	**Tolerance**	**p-level**
δF_{aq}	0.765671	0.288789	2.65131	0.021128
TBA-i	0.380253	0.164478	2.31187	0.039343

Table 3. Optimal linear and non linear regressions between individual parameters and time of chilled storage and TVB-N. Significance was declared at $P<0.05$. Abbreviations as specified in Table 1.

	TIME (days)		TVB-N	
	Model	**r**	**Model**	**r**
TVB-N	$a*\ln x$	0.65		
PV	$a+ 1/x +x^{1/2}$	0.84	$a*1/x$	0.32
CD	$a*x^3$	0.58	$a+1/x$	0.54
TBA-i	$a*x^{1/2}$	0.58	$a+b*x$	0.61
FFA	$a*x^2$	0.36	$a*x^2$	0.36
PI*	—	—	—	—
δF_{or}	$a*x^{1/2}$	0.46	none	0.00
δF_{aq}	$a*\ln x$	0.94	$a+b*x$	0.86
$\delta F_{or}/\delta F_{aq}$	$a*\ln x$	0.92	$a+1/x$	0.79

* No significant models could be found

Table 4. Multivariate non linear model to predict:

(4A) chilled storage time: Time (days) = $\alpha + \beta * \ln(\delta F_{aq}) + \lambda * (\text{TBA-i})^{0.5}$. Variance explained: 93.638 %

(4B) total volatile base-nitrogen. TVB-N = $\alpha + \beta * (\delta F_{aq}) + \lambda * (\text{TBA-i})$. Variance explained: 86.080 %

4A

	α	β	λ
Estimate	-7.68580	3.924000	4.699298
Standard Error	1.31119	0.624943	2.562429
Tolerance	-5.86171	6.278968	1.833923
p-level	0.00003	0.000015	0.086581

4B

	α	β	λ
Estimate	17.69096	0.129304	4.042041
Standard Error	1.21475	0.023632	5.397527
Tolerance	14.56342	5.471652	0.748869
p-level	0.00000	0.000064	0.465518

2