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# ORGANOLEPTIC AND CHEMICAL ASSESSMENT OF TWO FROZEN MARINE FISHES OBTAINED FROM MARKETS IN FOUR AGRICULTURAL ZONES OF OYO STATE, NIGERIA

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#### ABSTRACT

Sensory and non-sensory assessment of frozen fish: Sardinella species and Micromesistius poutassou obtained from four Agricultural Development Programme (ADP) zones (Ibadan/Ibarapa, Ogbomoso, Oyo and Saki) of Oyo State were carried out. Overall acceptability was based on 7-point Hedonic scale (very much liked (7) - very much disliked (1). Chemical tests [Hypoxanthine (Hx), Peroxide value (PV), Trimethylamine (TMA) and Free fatty acid (FFA)] were carried out using standard methods. Data were analyzed using the mean, standard deviation and t- test. Overall acceptability of the two frozen fish species was between  $5.47\pm0.20$  and  $5.90\pm0.08$ . Hx values varied between 22.15±1.04 and 25.96±0.53mg/100g fish for Sardinella spp. and M. poutassou respectively. The values recorded for both Sardinella spp. and M. poutassou were between 17.48±0.81 and 19.79±1.03 meq/kg for PV; 19.03±065 and 23.63±0.60 mg/100g fish for TMA and between 1.38±0.07% and 1.85±0.31% for FFA respectively. In both Sardinella spp. and M. Poutassou respectively, Oyo Zone had the least PV (17.48±0.81; 17.90±0.60meq/kg), TMA level (20.58±0.90; 19.03±0.65mg/100g). Sardinella spp. samples from the Ogbomoso Zone had highest PV (18.83±0.60meq/kg), FFA (1.85±0.13%) and TMA (23.63±0.60mg/100g) levels, and also the highest FFA values (1.81±0.19%) in M. poutassou. Hypoxanthine and TMA valueswere significantly different in the Sardinella spp. and M. poutassou samples across the zones, respectively. Quality of frozen fish was best in the Oyo Zone. All sensory and non-sensory indicators of fish quality were however within acceptable limits in the four zones of Oyo State.

Key words: Frozen fish, Sensory assessment, Chemical assessment, ADP zones, Oyo state.

# **INTRODUCTION**

In Nigeria, fish whether consumed as frozen, canned or cured represents a significant proportion of the animal protein in the diet. Fish consumption accounts for about 45% animal protein consumption in Nigeria because fish is less tough and more digestible compared to beef, mutton, chicken and bush meat (Cheke, 2014). Fish flesh is preferable because of a greater ratio of muscle protein of fish to the connective tissue (Oehlenschlager, 1997). Fish is relished because of the high proportion of polyunsaturated fatty acids of the (n-3) family and low cholesterol in its fat content (Sanchez-Alonso and Borderias, 2008).

Food and Agricultural Organization, FAO (2004), however reported fish quality, including safety as a major concern facing the food industry today. Quality deterioration of stored fish is inevitable with length of storage period (Jeon *et al.*, 2002). Spoilage changes result in the gradual accumulation in the flesh of compounds, the quantity of which provides a measure of the progress of spoilage that is independent of sensory assessment. Sensory assessment is the use of one or more of the five senses to judge or form an opinion on some aspects of quality. The senses in question are sight, smell, taste, touch and hearing (FAO/Codex, 1999). As fish spoils, it goes through a sequence of changes that are readily detectable by the human sense of sight, touch, smell and taste (Aitken et al., 1982). The spoilage compound is the trimethylamine (TMA) derived possibly partly by intrinsic enzymes, but certainly by bacteria action from trimethylamine oxide (TMAO) (Connell, 1995). Hypoxanthine is a degradation product of adenosine triphosphate and its level in a fish is an indication of the extent of spoilage (Kirk and Sawyer, 1999).

The energy intensive freezing operation is only a temporary method of preservation of fish as freeze storage can decrease microbial and enzymatic spoilage but cannot prevent oxidative spoilage (Ghaly *et al.*, 2010).During the frozen storage of fish, lipid hydrolysis and oxidation have been shown to occur (Verma *et al.*, 1995). These lipid reactions are known to cause rancidity, protein denaturation and texture changes which affect acceptability of the fish (Mackie, 1993).It has been proven that not all fat changes are mainly oxidative in nature. Some involve the hydrolytic liberation of fatty acids as well as other organic acids. Free Fatty Acid (FFA) formation as a result of lipid (triglyceride and phospholipid classes) hydrolysis has provided a suitable means for assessment of fish damage during frozen storage (Aubourg, 1999).

This study was undertaken principally to evaluate the sensory and chemical composition of the frozen fish sold in selected markets in Oyo State with the aim of predicting their suitability for human consumption.

# MATERIALS AND METHODS Sample Collection

Sardinella species (sardine) and Micromesistius poutassou (blue whiting) were selected from among the commercially important species of imported frozen fish sold in Oyo State. The two fish species were selected due to their availability, spread and acceptability to the consumers in all the four zones (Ibadan/Ibarapa, Ogbomoso, Oyo and Saki). Several samples of Sardinella species and Micromesistius poutassou with average body weight of 190g and length between 25-30 cm were collected from retail depots from these four zones used for this study. The samples were collected in suitable aluminum foil, placed in sterile plastic containers with ice and immediately taken to the appropriate laboratory for analysis. Sampling was done in the four zones once every two weeks in each of the zone over twelve month's period between January and December.

# Organoleptic Assessment

This assessment was carried out on both the uncooked and the cooked fish samples. Fish samples from different zones were presented bimonthly to a semi-trained five-man panel for organoleptic assessment in a well lit environment free of odour. The fish were presented to the taste panel immediately after purchase from retailers. Assessment was based on the scoring system by Minim, (2006) which involved measurement of certain parameters on a 7-point hedonic scale for the determination of selected characteristics. The seven categories were ranked as follows: very much liked (7), liked a lot (6), liked (5), liked and did not like (4), disliked (3), much disliked (2) and very much disliked (1).

For the cooked treatment, the fish samples were gutted and steamed whole for about 20 minutes; and allowed to cool to a comfortable tasting temperature. Samples were then presented to the taste panel on a clean plate. For the uncooked treatment, each fish sample was placed on a clean plate for assessment. Each assessor was given a scoring sheet to document their observation. The parameters assessed for the uncooked fish were: appearance or external characteristics (mucus, shape of the eye and rigidity of the abdominal wall, colour of the gills and skin, texture and odour). Cooked fish were assessed using only the texture, odour and taste.

## Sample Preparation/Analysis

The fresh frozen fish samples were blended using a sterilized blender and the blended samples were subsequently analyzed using standard method of AOAC (2002) to determine theHypoxanthine, Peroxide value, Free Fatty Acid and Trimethylamine.

Hypoxanthine was determined colorimetrically using 4-Dimethylaminobenzoic acid solution at a wavelength of 460nm.Crushed fish samples (2g) was weighed into a 250ml beaker, 1g of active carbon, 100ml distilled water and 5ml of Carrez solutions I and II were added and mixed for 30 minutes. The mixture was filtered through a Whatman No. 2 filter paper. Carrez solution I contain potassium hexacyanoferrate (II)trihydrate (K<sub>4</sub>[F(CN)<sub>6</sub>]x3H<sub>2</sub>O). Concentration: 15g/100ml. Carrez solutionsII contains zinc sulphateheptahydrate (ZnSO<sub>4</sub>x7H<sub>2</sub>O). Concentration: 30g/100ml. Concentration started from 0,2,4,6,8,10.

Peroxide value was volumetric method by titration of the liberated iodine with thiosulphate using starch as an indicator. Crushed fish sample (2g) was weighed into a 250ml beaker. 20ml of chloroform and 10ml of glacial acetic acid was added to the fish sample in the beaker and mixed. The mixture was filtered into 250 ml conical flask, 1ml of 5% (aq) saturated potassium iodide (KI) solution was added and shaken thoroughly. The homogenous mixture was placed on the hot plate to boil for 30 seconds. 25ml distilled water was added and shaken; 1ml of 1% starch was added and the hot mixture titrated against the 0.002M Na<sub>2</sub>SO<sub>3</sub>. A blank determination was also carried out at the same time.

Titrimetric method was used in determining Free Fatty Acid. Well- macerated fish sample (1g) was weighed into a 100ml beaker. 50ml of chloroform was added and stirred with a mechanical stirrer for 5 minutes to ensure a complete extract of fat from the fish flesh sample. The mixture was filtered through a Whatman No.1 filter paper into a 250ml conical flask. 25ml of the filterate was dissolved in 25ml of mixed neutral solvent (mixture of diethylether and alcohol neutralized with 0.1N NaOH at ratio 1:1. 1ml of 1% phenolphthalein solution was added and titrate against 0.1N NaOH until a pink colour, which persisted for 15 seconds was obtained.

%FFA=<u>VxNx282</u>x100 W

Trimethylamine was measured by colorimetric method using Resolic acid solution as indicator.A well ground fish sample (2g) was homogenized with 60ml of 5% TCA (Tetrachloroacetic acid). The mixture was properly homogenized to obtain uniform slurry. The slurry was filtered into a 50ml volumetric flask to obtain a clear filtrate. 5ml of the clear filtrate was pipetted into a semi-micro distillation apparatus to which 5ml of 2M NaOH was added. The mixture was then steam-distilled in the distillation apparatus into 15ml of 0.01M HCl solution in 50ml conical flasks. 1ml of Resolic acid indicator solution was added to give a bluish colour. The mixture was then titrated to give a pale pink end point with 0.01M NaOH solution to obtain V<sub>1</sub>. Note that to every 10ml liquid in the titration flask, 1ml of 16% neutralized formaldehyde solution was added. 1ml of 16% formaldehyde (neutralized) was added to the mixture in the titration flask to liberate excess acid. The excess acid was titrated with 0.01M NaOH to obtain  $V_2$ 

$$TMA = \frac{14 (300 + weight of sample taken) \times V_2}{Nitrogen 500}$$

#### **Statistical Analysis**

The statistical programme, (SPSS, 2003) VERSION 16.0 was used to analyse the result of the treatments. Descriptive statistics were used to analyze the data collected while T-test was used to determine the differences in the quality parameters of the two frozen fish species examined.

# RESULTS

# **Sensory Evaluation**

The mean hedonic scores obtained for the taste, odour, texture, appearance, colour and overall acceptability of the two fish samples are presented in Table 1. Sardinella spp. samples from Oyo Zone had the least values in taste  $(5.67\pm0.21)$ , odour  $(5.67\pm0.21)$ , texture  $(5.50\pm0.22)$ , appearance  $(5.33\pm0.33)$  and colour  $(5.17\pm0.40)$  across the four zones. The highest values for taste  $(6.17\pm0.30)$  and colour  $(5.83\pm0.16)$  was recorded in Saki Zone, Ogbomoso Zone in texture  $(6.00\pm0.25)$  while values for appearance  $(5.67 \pm 0.21)$  and odour  $(5.83 \pm 0.16)$  were highest in both Ogbomoso and Saki zones respectively. M. poutassou recorded the lowest values in taste  $(5.75\pm0.25)$  and odour  $(5.67\pm0.18)$  from fish samples collected from Ibadan/Ibarapa Zones, while the lowest values for texture, appearance and colour were 5.50±0.22 (Ogbomoso and Oyo), 5.33±0.21 (Ogbomoso) and 5.00±0.22 (Saki) respectively. The highest value for taste  $(6.17\pm0.16)$  was recorded for *M. poutassou* samples in Ogbomoso Zone, while Saki Zone had highest value in odour (6.00±0.25), texture (5.67±0.21), appearance  $(5.67 \pm 0.21)$  and colour  $(5.83 \pm 0.16)$ across the four zones respectively. The mean acceptability values were within a range of 5.47±0.20 (Oyo) - 5.90±0.08 (Saki) recorded for Sardinella spp. and 5.57±0.13 (Oyo) - 5.80±0.10 (Saki) for *M. poutassou* respectively. However, there were no significant differences (p>0.05) between the mean acceptability scores recorded for the two species across the zones (Table 3).

Physical				Zone	Zone				
parameters	Ibadan/ Ibarapa		Ogbomoso		Оуо		Saki		
	Sardinella spp.	M. Þoutassou	<i>Sardinella</i> spp.	M. poutassou	<i>Sardinella</i> spp.	M. poutassou	<i>Sardinella</i> spp.	M. poutassou	
Taste	5.83±0.16	5.75±0.25	6.17±0.16	6.17±0.16	5.67±0.21	6.00±0.25	6.17±0.30	$5.83 \pm 0.30$	
Odour	$5.75 \pm 0.17$	$5.67 \pm 0.18$	$5.83 \pm 0.16$	$5.67 \pm 0.21$	$5.67 \pm 0.21$	$5.83 \pm 0.16$	$5.83 \pm 0.16$	$6.00 \pm 0.25$	
Texture	$5.67 \pm 0.14$	$5.67 \pm 0.18$	$6.00 \pm 0.25$	$5.50 \pm 0.22$	$5.50 \pm 0.22$	$5.50 \pm 0.22$	$6.00 \pm 0.00$	$5.67 \pm 0.21$	
Appearances	$5.50 \pm 0.23$	$5.67 \pm 0.18$	$5.67 \pm 0.21$	$5.33 \pm 0.21$	$5.33 \pm 0.33$	$5.50 \pm 0.22$	$5.83 \pm 0.16$	$5.67 \pm 0.21$	
Colour	$5.25 \pm 0.25$	$5.42 \pm 0.22$	$5.33 \pm 0.42$	$5.33 \pm 0.21$	$5.17 \pm 0.40$	$5.00 \pm 0.00$	$5.67 \pm 0.21$	$5.83 \pm 0.16$	
Overall									
Acceptability	$5.60 \pm 0.15$	$5.63 \pm 0.11$	$5.80 \pm 0.12$	$5.60 \pm 0.13$	$5.47 \pm 0.20$	$5.57 \pm 0.13$	$5.90 \pm 0.08$	$5.80 \pm 0.10$	

**Table 1.** Mean Hedonic scores for Sardinella spp. and Micromesistius poutassou samples across thezones in Oyo State

#### **Chemical Evaluation**

# Peroxide Value and Free Fatty Acid Concentration

Results indicated that frozen fish samples obtained from Oyo Zone had the lowest peroxide values in both the *Sardinella* species  $(17.48\pm0.81 \text{meq/kg})$  and *M. poutasson*  $(17.90\pm0.60 \text{meq/kg})$ . Frozen fish samples from Ogbomoso Zone had the highest PV  $(18.83\pm0.60 \text{meq/kg})$  for the *Sardinella* spp. compared to *M. poutassou* samples from Saki Zone  $(19.79\pm1.03 \text{meq/kg})$ . The peroxide concentration in the frozen fish samples was generally lower for the *Sardinella* spp. obtained from the four zones than *M. poutassou* samples (Table 2).

Ogbomoso Zone had the highest mean concentration of FFA ( $1.85\pm0.31\%$ ;  $1.82\pm0.19\%$ ) in both the *Sardinellaspp*. and *M.poutasssou* respectively. The lowest mean FFA value was however recorded in the samples collected from the Ibadan/Ibarapa Zone ( $1.52\pm0.14\%$ ;  $1.38\pm0.07\%$ ) respectively. *Sardinella* spp. had higher FFA values in Ibadan/Ibarapa, Ogbomoso and Oyo zones when compared with *M. poutassou*. The only exception was recorded in the samples of the species collected from the Saki Zone.

#### **Trimethylamine level**

The mean concentration of TMA in the two species by zones (Table 2) showed that frozen fish samples of *M. poutassou* from Ibadan/Ibarapa

Zone had the highest concentration of TMA (22.96 $\pm$ 0.49 mg/100g), followed by the samples of the fish from Saki Zone (21.79 $\pm$ 3.32 mg/100g) while the least values was recorded in the fish sample collected from the Oyo Zone (19.03 $\pm$  0.65 mg/100g). In *Sardinella* spp., the highest concentration of TMA was recorded in samples of the fish collected from Ogbomoso Zone (23.63 $\pm$ 0.89 mg/100g) while the lowest (20.58 $\pm$ 0.90 mg/100g) was recorded in the samples from the Oyo Zone.

#### Hypoxanthine Level

Hypoxanthine levels were higher inM. Poutassou samples collected from all the zones compared with those of Sardinella spp. The highest hypoxanthine values for both Sardinella spp. and *M. poutassou* were recorded in frozen fish samples collected from the Oyo Zone  $(25.54 \pm 0.41 \text{mg}/100 \text{g} \text{ and } 25.96 \pm 0.53 \text{mg}/100 \text{g})$ respectively. The lowest hypoxanthine level  $(22.15\pm1.04 \text{ mg}/100\text{g})$  in Sardinella spp. samples was recorded from Saki Zone and for M. poutassou samples (25.49±0.65 mg/100g) collected from Ibadan/Ibarapa Zone. The mean values of the quality indices in the two fish species (Table 3) showed that there were no significant differences (p > 0.05) in the organoleptic parameters, PV, TMA and FFA levels. However, the mean hypoxanthine levels between the two species was significantly different (p < 0.05) from each other.

Fish species	Zones	PV ( meq/kg)	TMA (mg/100g)	FFA (%)	Hx (mg/100g)
<i>Sardinella</i> spp.	Ibadan/Ibarapa Ogbomoso	$18.52 \pm 0.55$ $18.83 \pm 0.60$	$23.07 \pm 0.65 \\ 23.63 \pm 0.60$	$1.52 \pm 0.14$ $1.85 \pm 0.31$	$24.48 \pm 0.56$ $24.47 \pm 0.77$
·rr	Oyo Saki	$17.48 \pm 0.81$ $18.50 \pm 1.69$	$20.58 \pm 0.90$ $22.79 \pm 3.02$	$1.73 \pm 0.27$ $1.53 \pm 0.17$	$25.54 \pm 0.41$ $22.15 \pm 1.04$
Micromesistius Poutasssou	Ibadan/Ibarapa Ogbomoso Oyo Saki	19.47±0.42 19.56±0.75 17.90±0.60 19.79±1.03	$22.96 \pm 0.49$ 19.89 ±1.66 19.03 ± 0.65 21.79 ±3.32	$1.38\pm0.07$ $1.82\pm0.19$ $1.69\pm0.22$ $1.67\pm0.19$	25.49±0.65 25.69±1.01 25.96±0.53 25.77±0.49
International Standards		10-20 meq/kg	30mg/100g	1.85%	29.58mg/100g

**Table 2.**Mean concentration of chemical parameters in *Sardinella* spp. and *M. poutassou* samples across Zones in Oyo State.

Table 3.T- Test for Mean values of Quality indices in *Sardinella* spp. and *M. poutassou* samples across Zones in Oyo State.

Parameter	Fish species	Ν	$\bar{x} \pm SEM$	Df	t-value	P Level
Organoleptic	Sardinella spp.	30	$5.67 \pm 0.08$	58	0.26	0.795
	M. poutasssou		$5.65 \pm 0.06$			
Peroxide value( meq/kg)	<i>Sardinella</i> spp.	30	18.38±0.43	58	-1.58	0.120
	M. poutasssou		19.24±0.34			
Trimethylamine $(mg/100g)$	Sardinella spp.	30	22.63±0.69	58	1.48	0.145
	M. poutasssou		21.33±0.55			
Free fatty acid (%)	Sardinella spp.	30	$1.63 \pm 0.11$	58	0.30	0.768
	M. poutasssou		$1.59 \pm 0.08$			
Hypoxanthine(mg/100g)	Sardinella spp.	30	$24.23 \pm 0.40$	58	-2.78	0.007*
	M. poutasssou		$25.68 \pm 0.35$			

\*Significantly different at p < 0.05

# DISCUSSION

The organoleptic properties of the examined frozen *Sardinella* spp. and *M. poutassou* samples collected from the four zones of Oyo State indicated that the products were acceptable according to the panel's evaluation using 7-point Hedonic scale designed by Minim(2006).

Lakshmanan (2000) posited that the acceptable limitof Peroxide value in fish should be between 10-20 meq/kg values, above which afish would probably smell and taste rancid. However, the peroxide values for frozen fish recorded across the four zones of the state fell within this limit.Frozen fish from the Oyo Zone with the lowest PV of 17.48 and 17.90 for both *Sardinella* spp. and *M. poutassou* respectively, had the lowest tendency to go rancid.In the same vein, Free Fatty Acid concentration in both frozen fish samples from across the zones, were found to be within the acceptable limit of 1.8% established by Oyelese (2012).Trimethylamine according to Connell and Shewan (1980)is a well accepted indicator of freshness or spoilage in marine fishes. The TMA level in the two frozen fish samples across zones of Oyo State fell within the acceptable limit of 30mg/100g fish recommended by Regenstein *et al.* (1982).

Hypoxanthine is an important parameter used for

estimation of wholesomeness or otherwise of fishes. It begins to accumulate shortly after the death of fish and it increases with storage time (Nakatami et al., 2005). Hypoxanthine level in the two frozen fish samples from the four designated zones of the state wasalso within acceptable limits of 29.58mg/100g fish as reported by Oyelese (2012). The results obtained agreed with previous studies by Howgate (1982), Watanabe et al. (1983), Yao (1993), Niu and Lee (2000). Theauthors reported that hypoxanthine concentration increases with storage time making it a good predictor of spoilage over a wide range of quantities and it is best suited for a wide range of species products than both TMA and total volatile base (TVB). Metin et al. (2001) also stated that organoleptic spoilage became obvious at the highest level of hypoxanthine. In the present studyhowever, the significant difference (p < 0.05) in the total mean concentration of hypoxanthine levels in the two fish species across zones marked differences between Sardinella spp. and M. poutassou. All the chemical parameters (Hx, PV, FFA and TMA) assayed, which were within the acceptable limits, probably reflected the high overall acceptability of the two frozen fish species in Oyo State.

### CONCLUSION

The organoleptic properties of the examined samples from the two species showed thatthe fishes were acceptable in Oyo State. However, detailed chemical studies provedthat the two frozen fishes had the best keeping quality in the Oyo Zone,while frozen fishes from Ogbomoso Zone had the least quality amongst the four zones in the state.

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# REFERENCES

- Aitken, A., Mackie, I.M., Merrit, J.H. and Windsor, M.L. 1982. Fish Handling and Processing. HMSO Edinburgh. Pp 167-186.
- AOAC. 2002. Official Methods of Analysis of the Association of Official Analytical Chemists (17<sup>th</sup> Edition). Virginia. 1298pp.

- Aubourg, S.P. 1999. Lipid damage detection during the frozen storage of an underutilized fish species. *Food Research International*, 32 (7): 497-502.
- Cheke, A. O. 2014. Markets and marketing of fish and fishery products in Nigeria.*IIFET* 2014 Australia Conference Proceeding.
- Connell J.J. 1995. *Control of FishQuality*.4th edition. Farnham, England. 245pp.
- Connell J.J. and Shewan J.M. 1980.Past, present and future of fish science. Advances in Fish Science and Technology. Fishing news book. Pp30-63.
- FAO/Codex 1999. Guidelines for the sensory evaluation of fish and shellfish laboratories. CAC/GL 31:3-33.
- Food and Agriculture Organization (FAO). 2004. Assessment and management of seafood safety and quality. *Fisheries Technical Paper* 444. Pp1.
- Ghaly, A.E., Dave, D., Budge, S and Brooks, M.S. (2010).Fish Spoilage Mechanism and preservation Techniques: Review.*American Journal of Applied Sciences*. 7(7):859-877.
- Howgate, P.F. 1982. Fish handling and processing. *Torry Research Station*. HMSO Press.Pp20-42.
- Jeon, Y.J., Kamil, J.Y. and Shahidi, F. 2002. Chitosan as an edible invisible film for quality preservation of Herring and Atlantic cod.*J. Agric. Food Chem.* 50(18): 5167-5178.
- Kirk, S.R and Sawyer, R. 1999. *Pearson's composition* and analysis of foods. Edn. Longman, UK. 9<sup>th</sup> Edition.Pp 504-525.
- Lakshmanan, P.T. 2000. Fish spoilage and quality assessment. In: Quality Assurance in Seafood Processing T.S.G. Iyer, M.K. Kandoran, M. Thomas, and P.T. Mathew. Eds. Cochin: Society Fisher Techno., India. Pp 26-40.
- Mackie, I. 1993. The effects of freezing on flesh proteins. *Food Reviews International* 9: 575-610.
- Metin, S., Erkan N. and Varlik, C. 2001. The application of Hypoxanthine activity as a quality indicator of cold stored fish burger.Research article, Istanbul University, Turkey.
- Minim, V.P.R. 2006. *AnaliseSensorial: Estudo Com Consumidores*. Vicosa: Editora da Universidade Federal de Vicosa. 225pp.
- Nakatami, H. S., Santos, L.V., Pelegrine, C.P.,

Gomes, S.T., Matsushita, M., Sousa, N.L and Visentainer, J.V. 2005. Biosensor based on Xanthine Oxidase for Monitoring hypoxanthine in fish meat. *American Journal of Biochemistry and Biotechnology*. 1 (2):85-89.

- Niu, J and Lee, J.Y. 2000. Bulk modified amperometric biosensors for hypoxanthine based on sol-gel technique. *Sensor Actuat. B. Chem.* 62:190-198.
- Oehlenschlager, J. 1997. Marine fish- A source for Essential Elements? In: Seafood from Producer to Consumer, Integrated Approach to Quality. Luten, J.B., T. Borresen and J. Oehlenschlager Eds. Elsevier Science Publishers B.V. Amsterdam, Netherlands. Pp 641-652.
- Oyelese, O.A. 2012. Hypoxanthine levels, chemical studies and bacterial flora alternate frozen/thawed market simulated marine fish species. *Progress in food preservation*.1<sup>st</sup> Edition.
- Regenstein, J.M., Schlosser, M.A., Samson, A. and Fey, M. 1982.Chemical changes of trimethylamine oxide during fresh and frozen storage of fish.*In: Chemistry and*

Biohemistry of marine Food Products. R.E. Martins, G.J. Flick, C.F. Hubard and D.R. Ward. Eds. Westport, Connecticut, 137-148.

- Sanchez-Alonso, I and Borderias, A. 2008. Technological effect of red grape antioxidant dietaryfibre added to minced fish muscle.*Journal of Food Science and Technology*. 43: 1009-1018.
- SPSS. 2003. Statistical package for social science for Windows 16. SPSS Inc., Chicago, IL, and USA.
- Verma, J., Srikar, L., Sudhakara, N. and Sarma J. 1995. Effects of frozen storage on lipid freshness parameters and some functional properties of oil sardine (*Sardinellalongiceps*) mince. *Food Research International*, 28: 87-90.
- Watanabe, E; Ando, K., Karube, I.; Matsuoka, H and Suzuki, S. 1983. Determination of hypoxanthine in fish meat with an enzyme sensor.*J. Food Sci.* 48: 496-500.
- Yao, T. 1993. Enzyme electrode for the successive detection of hypoxanthine and inosine. *Anal. Chim. Acta*. 281:323-326.