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Effects of temperature and humidity and effectiveness of some selected antioxidants on lipid oxidation of fresh nile tilapia (*Oreochromis niloticus* L.) of Lake Victoria, Kenya

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Abstract

Spoilage of fish and its related products due to oxidation is a primary problem leading to both loses in income and environmental degradation. Various methods such as sun-drying, smoking and refrigeration as currently used to prevent such loses are either costly, unavailable to the intended users or ineffective. In addition, the anti-oxidants available in the market to mitigate loses occasioned by oxidation to the fish are mainly artificial and yet to be optimized for Oreochromis niloticus. This study quantified the interactive effects between the storage temperature and ambient humidity and the effectiveness of Citric acid, Vitamin E (tocopherol), Rosemarinic acid, Propyl gallate and Vitamin C in arresting fish tissue oxidation. This study observed the fish, starts to rot from the head and this spoilage could only be controlled by temperature at low to medium humidity (< ca. 35 %) values. At high humidity, ca. 65%, the rate of spoilage was purely humidity controlled. The effectiveness order of Vitamin E ≥ Rosmarinic acid > Propyl gallate >Citric acid > Vitamin C was realized and their molecular rotatable bond counts and the kinetic diameters were the most influential properties. Thus, for effective storage, separating the head from the rest of fish and doing the refrigeration in less humid environments is recommended. The relative efficacy of the anti-oxidants could be predicted by evaluation of their relative rotatable bond counts and the kinetic diameters.

Keywords: Oxidation, rancidity, natural anti-oxidants, temperature, humidity.

Introduction

In 1996, the World Health Organization (WHO) affirmed that access to nutritionally sufficient and safe food is a right of every person; yet, despite improvements in technology, food free from contamination, spoilage, and diseases remains an unresolved challenge in both the agricultural and food industries[1]. In particular, sicknesses due to unsafe foods is arguably one of the leading health problem worldwide and an import unite explanation for reduced economic productivity[1,2]. The most challenged are communities whose livelihood revolves around fishing, a known highly perishable food. Generally, seafood products contain highly unsaturated fatty acids that are prone to oxidation rendering the products highly susceptible to rancidity. The reaction, as graphically described in Fig. 1, is postulated to be a chain reaction involving the initiation, propagation and termination steps. Oxygen is evidently a critical requirement in lipid oxidation (Figure-1). Generally, lipid oxidation begins from the surface of the food material to the interior as the oxygen that induces the process diffuses into the food substance. Traditionally, antioxidants are incorporated to the food system to cutoff oxygen supply thus preventing lipid oxidation. Incorporation of antioxidants all through a food matrix is unnecessary since oxidation begins at the exterior as the interior fraction of a whole unbroken food muscle is normally anaerobic[3]. It is, therefore, viable to ameliorate preservation by adding the antioxidants on exterior surfaces of the food only since this cuts off the oxygen supply needed for oxidation process to take place[4].

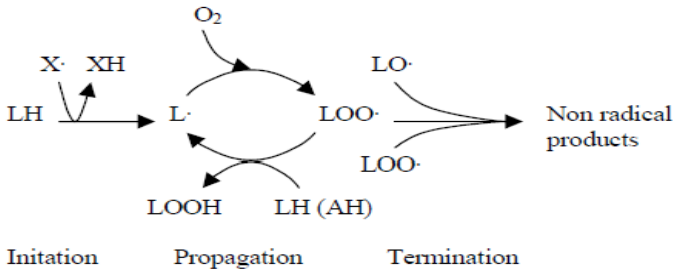


Figure-1: Schematic illustration of lipid oxidation process. LH = unsaturated lipid, X^o= initiator, L^o = alkyl radical, LO^o = alkoxyl radical, LOO^o = peroxy radical, LOOH = hydroperoxide, and AH = anti-oxidant. Adopted from Huang et al[4].

The processing of fish and fish products necessarily includes a storage period for the processed product(s) before sale or consumption. Since fish is a highly perishable product, the duration of storage should be minimized with suitable conditions availed to inhibit deteriorative oxidative damage and microbial or pests infestation. Among the most significant environmental parameters affecting the shelf-life of fish and its products are temperature and humidity since they drive the microbial flora activity and the rate of chemical changes[5]. However, the synergistic or antagonistic interaction between temperature and humidity is yet to be reported.

Oxidation of fish lipids results in development of undesirable odour and off-flavours that shorten the shelf-life of fish and related products and limit their utilization in seafood-based products[6]. These losses affect consumer acceptability, commercial value and income tofishermen and traders. Furthermore, the undesirable health implication associated with consumption of spoilt fish and the attendant environmental impact cannot be gainsaid. The net result is decrease in the nutritional content available to the consumers.

Several methods have been developed to preserve fish products by limiting lipid oxidation. For instance, incorporation of tripolyphosphates, ascorbate, and phenolic antioxidants into Atlantic mackerel (surimi) were effective in the control of lipid oxidation[7]. Smoking is one oldest fish processing strategies used to prevent or limit post-harvest losses. Additionally, at industrial levels, vacuum packaging, controlled packaging atmosphere with carbon dioxide, and oxygen absorbers are predominantly used toimpede lipid oxidation[6]. Although the above mentioned techniques can reduce the lipid oxidation in fish, they also have some limitations associated with artificial food additives and the inherent high costs involved.

The effective amount of synthetic antioxidant required to manage the losses due to lipid oxidation can be reduced by it being applied on the food surface. Direct application of artificial food additives to minimize deterioration and maintain nutritional value has also risen substantially over the past 25 years. However, as Timbo et al. observed, the benefits provided are not without potential risk[8]. Challenges associated with application of antioxidants solely on food surfaces include the safety of the solvent used for dilution of the antioxidant and the efficacy of the technique used for applying the diluted antioxidant media onto the food surface. Furthermore, once the packaging is materially compromised or destroyed during storage, transport, sale point, or opened by end users, the anti-oxidative properties are lost. Any residual oxygen present in the tissue may also to trigger lipid oxidation despite vacuum packaging. As such, additional antioxidants are incorporated into the processed foods to limit lipid oxidation from destroyed packaging. Therefore, the use of antioxidants is inescapable regardless the nature of anti-oxidation system employed to control lipid oxidation.

Generally, lipid oxidation is considered the principal reaction accounting for quality deterioration of fish products. Food qualities affected by lipid oxidation include flavor, colour, texture and its nutritional value. Specifically, Clucas[9] observed that the shell-life of fish depends on handling during processing, acidity level, species of fish, weather conditions, storage method and temperature during distribution. Chemical breakdown of protein, fat and tissue water contents all contribute to quick spoilage of fish[10].

The identification and proper utilization of safe natural food preservatives is therefore a priority in order to provide adequate and safe food supply. In addition, these anti-oxidants, mainly from leafy spices and herbs (Table-1), possess anti-carcinogenic effects and inhibit biologically harmful reactions *in vivo*[11]. Many natural plant extracts primarily contain phenolic compounds which are potent antioxidants[12]. In addition, certain phenolic compounds for example rosemary, tea cloves, hops, basil, thyme and coriander have been demonstrated to have antimicrobial activity against food borne pathogens[13]. There are several antioxidants in the market but most of them are synthetic. Natural antioxidants are considered safer and are not carcinogenic. They can therefore be applied directly onto the fish. It is on this background that this study set to document the efficiency of natural antioxidants in controlling lipid oxidation in muscles of fresh water fish. The Nile tilapia (*Oreochromis niloticus* L.) was chosen for this study partly because it is the third most important fish species in Lake Victoria (East Africa) and one of the most preferred by consumers.

This study tested the effectiveness of selected natural antioxidants in controlling lipid oxidation in fresh *O. niloticus*. The natural antioxidants used were: Citric acid (2-hydroxy-1, 2, 3-propane-tricarboxylic acid), Vitamin E (tocopherol), rosemary *Rosmarinus officinalis* L. (Labiatae), Propyl gallate (PG) and Vitamin C (Ascorbic acid - 3-oxo-L-guiofuranolactone).

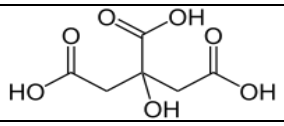
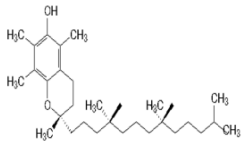
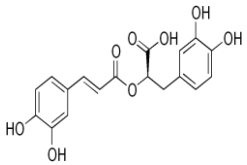
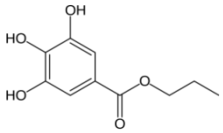
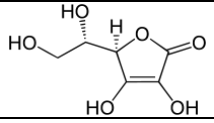
Citric acid is highly soluble in water but insoluble in fats. It is efficacious in inhibiting oxidative deterioration of lipids and is therefore widely applied to vegetable oils after deodorization[15,16]. Vitamin E (tocopherol) is the natural antioxidant mixture of alpha, gamma, and delta tocopherols. Tocopherols are considered the most significant natural antioxidants derived from vegetable oil-based foods. However, tocopherols may exert a pro-oxidant effect at high concentrations and their activity is temperature-dependent[11]. The antioxidant properties of rosemary have been exploited for centuries[17]. Several phenolic compounds with antioxidants activities have been isolated, characterized and identified from rosemary leaves[6] and the most commonly known is rosmarinic acid. Propyl gallate (PG) is an aromatic compound which reduces lipid oxidation and rancidity during long freezing storage[16,18]. Nicolalde et al.[19] showed that exposure of rib bones to PG decreased visible discoloration during storage in a high oxygen environment. Vitamin C is highly insoluble in fats and was first applied as an antioxidant to improve the stability of

mayonnaise[20]. Ahn and Nam[21] reported that ascorbic acid is a reducing agent, inhibiting myoglobin oxidation and development of brown color in non-irradiated beef.

Among the properties of anti-oxidant compounds that affect their reactivity and surface adherence and were investigated in this study are: i. pK_a which indicates how acidic (or not) a given hydrogen atom in a molecule is. Usually, the higher the pK_a and the stronger the acid and more available is the Hydrogen for reaction. This property would tend to reduce the oxidative process in a molecule, ii. The number of rotatable bonds (RBN) is the number of bonds which allow free rotation around themselves. These are defined as any single bond, not in a ring, bound to a non-terminal heavy atom. This flexibility would be a bonus when a molecule is to access a restricted reaction site. Iii. The kinetic diameter (k_d) has also been correlated with the molecular weight using the following equation for aromatic hydrocarbons, i.e., $d = 1.234(MW)^{1/3}$, Where MW is the molecular weight in g mol^{-1}. This kinetic diameter estimation postulates a spherical molecule and translates to the area covered by the molecule in a surface restricted reaction[22], iv. The number of acceptor atoms for H-bonds (nHAcc) is a measure of the hydrogen-bonding ability of a molecule expressed in terms of number of possible hydrogen-bond acceptors. Specifically, it is relevant as a sink for radical hydrogen species and v. The polar surface area (PSA) of a molecule is the surface sum over all polar atoms, especially oxygen and nitrogen including their attached hydrogens. PSA is widely applied in optimization of a drug's capacity to permeate cells. Molecules possessing a polar surface area greater than 140$Å^2$ are generally poor at permeating cell membranes. In this case, it will be relevant in determining whether the anti-oxidation activity is a surface or a bulk-of-tissue phenomenon.

The potential use of these natural preservatives may assist in the development of novel, healthy anti-oxidative preservative regimes tailored towards organoleptic quality needs of specific natural food products. Consumers are demanding higher quality products that are appetizing, minimally processed and free of artificial preservatives but with an extended shelf-life. Spoilt fish produces a repulsive smell due to the amines and contributes to the contamination of the environment in addition to being harmful to humans if consumed. This study aimed to document the spoilage-time progression at ambient conditions the minimum maintainable temperature and moisture content that will prevent spoilage of fish and to evaluate the chemical efficacy of the natural anti-oxidants. Also, because the reaction as depicted in Figure-1 requires that the radicals generated at the initiation step be stable until the termination step, the role of both physical and chemical properties of the anti-oxidant molecules (Table-1) were also investigated.

Table-1: Chemical and Physical properties of the anti-oxidants.

General Name	Chemical structure	Lowest pKa (Strong acids)(a.u)	Rotatable bond counts (a.u)	Kinetic diameter (Å)	Hydrogen count (acceptor) (a.u)	Polar Surface Area ($Å^2$)
Citric acid		3.08	5	7.12	7	132
Vitamin E (tocopherol)		10.8	12	9.32	2	48.4
Rosmarinic acid		3.13	7	8.78	7	145
Propyl gallate		8.11	4	7.36	5	87
Vitamin C(Ascorbic acid - 3-oxo-L-guiofuranolactone).		4.36	2	6.92	5	101

(Adapted from Drugbank[14]; a.u = arbitrary units).

Materials and methods

The Nile tilapia (*Oreochromis niloticus*) fish bred from a brood stock from Lake Victoria used in this study were obtained fresh on the day of the experiment from the Maseno University fish pond. Each fish specimen was sliced into three 10g portions from the head, mid-section and tail respectively. The sliced portions were placed in the incubator at various isothermal storage temperatures (4°C to 40°C) and at controlled humidity levels of low (20±5%), medium (35 ± 8%), and high (65±10%) under aerobic conditions for 0.0, 0.5, 1.0, 1.5, 2.0, 4.0 6.0, 8.0, and 20.0 hours' time interval after which the samples were removed for analysis. For every temperature at 4, 20, 30, and 40^{o}C, effects of three (3) combinations of humidity; low, medium, and high were monitored. The low humidity was achieved by introducing anhydrous Calcium chloride into the setup. The medium humidity was the normal humidity of the day (35±8%), while high humidity was generated by saturating cotton wool with distilled water. For each specific temperature and humidity, 3 portions of the fish were used: head, mid-section and tail. The effective concentration of the anti-oxidant was also investigated at 0.0, 0.5, 1.0, 1.5, and 2.0g of anti-oxidant per 50mL distilled water. The experiments were done in triplicate.

The quantification of deterioration levels in the fish was done following the method by Lemon[23]. A sodium phosphate buffer was first prepared by mixing 3.45gL^{-1} sodium phosphate monobasic monohydrate 3.55gL^{-1}sodium phosphate dibasic anhydrous, 1gL^{-1}EDTA and 1gL^{-1} N-propyl gallateto form a sodium phosphate buffer solution. Trichloroacetic Acid solution (TCA) was also prepared by dissolving 75g of Trichloroacetic Acid in 50mL distilled water. A TCA solution buffer (8:2) was then prepared by combining 80mL of sodium phosphate buffer solution prepared with 20mL of TCA solution. In the next step, 20M Thiobarbituric acid (TBA) was prepared by dissolving 1.441g of 2-Thiobarbituric acid in 500mL distilled water. A Malonaldehydebis (diethyl acetal) (MBA) stock solution was prepared by dissolving 220.3mg in water to give 2.203gL^{-1}MBAsolution. 1mL of the stock solution was dissolved in 1L of distilled solution to form the standard solution. 0, 0.5, 1.0, 1.5, 2.0, and 2.5mL aliquots of MBA standard solution were then pipetted into screw capped test tubes. The total volume of each tube was brought to 4mL by adding the buffer TCA. The tubes were for the MBA standard curve.

0.1g of each of the fish samples to be analyzed were weighed accurately into screw capped test-tubes in triplicate. 4mL of buffer TCA solution was added to each tube, capped and vortexed for 15 seconds. 4mL of TCA preparation was added to each test-tube including those for the standard curve. A blank was prepared using 2mL distilled water and 2mL TBA solution. All the tubes containing the standards and the samples were placed in a boiling water bath for 20 minutes after which they were removed and cooled rapidly. The absorbance of the solutions was read at 533nm in UV-Vis spectrophotometer. Regression analysis was used to reveal the effects of time, humidity and temperature on the interactions of the anti-oxidants with the fish tissues.

Results and discussion

At first, an understanding was sought on the lipid oxidation of fish under normal room temperature (25.0±2.0^{o}C), Pressure (1 Atm.), and humidity (35±8%). Lipid oxidation in fish results in production of Malonaldehyde (MBA). MBA reacts with Thiobarbituric acid to yield a colored compound that can be measured spectrophotometrically. The more the MBA produced, the greater the lipid oxidation. The decay graph (Figure-2) details the changes observed as measured by decay indicator of MBA for the various parts of fish. The obtained S-shaped curves are typical of lipid oxidation progressive curves and are consistent with a self-propagating reaction as depicted in Figure-1[4]. On average, the production of MBA peaked by the 6th hour. Figure-2 indicates that, under room conditions, the head section of the fish is more susceptible to lipid oxidation, followed by the middle section and lastly the tail. The head section is more exposed to the environmental conditions due to the high internal surface areas offered by several organs openings. In addition, it is also where the highest concentration of the omega 3polysaturated fatty acids is found. This exposure of the head is followed by the middle section which consists of fewer organ openings. Thus it is not unexpected when in terms of levels of lipid oxidation, the middle region was second to that of the head. The tail being imbued with the least number of organs exposed to the surface offers limited internal surface area exposed to the atmospheric conditions and as such was least in lipid oxidation.

Bataringaya[24] reported a similar trend in oxidation of refrigerated Cod at 0°C and his values peaked on the 6th Day. In Figure-2, the on-set of physical signs of spoilage of soft flesh and milky pupils[25] became evident at 3 hr. By the 6th hour, the strong oduor of rotten fish could be detected within the room. Thus the physical spoilage would be equated to about 0.08 a.u. MBA, while the off-odour begins to be detected at MBA $\geq$ 1.0. An earlier study by Onyango et al.[26] showed that the community around Lake Victoria considers that, under normal atmospheric conditions, fish begins to get spoilt in ca. 6 hrs. However, Briggitte et al.[25] after exploring several methods of preservation, reported that in high ambient temperatures of the tropics, fish gets spoilt within 12 hours. Chemical methods for determination of freshness quality are arguably highly objective and therefore superior relative to methods dependent on sensory evaluation[27]. The disadvantage of non-sensory methods; biological, chemical, and physical, is the associated complexity since they require laboratory equipment[28]. In this study, with an MBA value of 0.13a.u., the smell of the rotting fish could be detected from 6 hours.

From Figure-2, the production of MBA levels off at ca. 3 hours. Consequently, all our time bound studies such as effects of

Temperature, humidity levels and amounts of anti-oxidants as presented in Figure-3, 4, and 5 and Tables 2 and 3 were considered at 3 hours' exposure time.

Generally, in Figure-3, high humidity (ca. 65%) encouraged the oxidation of the fish even at very low temperatures (4 – 20 ^{o}C). At both the medium (ca. 35%) and the low (ca. 20%) humid conditions, spoilage effects were somewhat dependent on rise in temperatures. This seems to agree with Briggitte et al.[25] who observed that higher water contents in food tissues or higher ambient humidity conditions encourages food spoilage. Specifically, beyond some critical levels of water in the food tissues, bacterial growth is supported resulting in higher fixing or availability of oxygen for oxidation processes. However, Antolovich et al.[3], additionally informs that oxidation mechanisms can vary significantly with rise in temperature. Thus from Figure-3, it would be advisable that lower ambient humidity is desired for better refrigeration results on fish.

An understanding was then sought for the synergistic interactions between the ambient humidity and the temperature for each body part of the fish sample and the findings are given in Table-2.

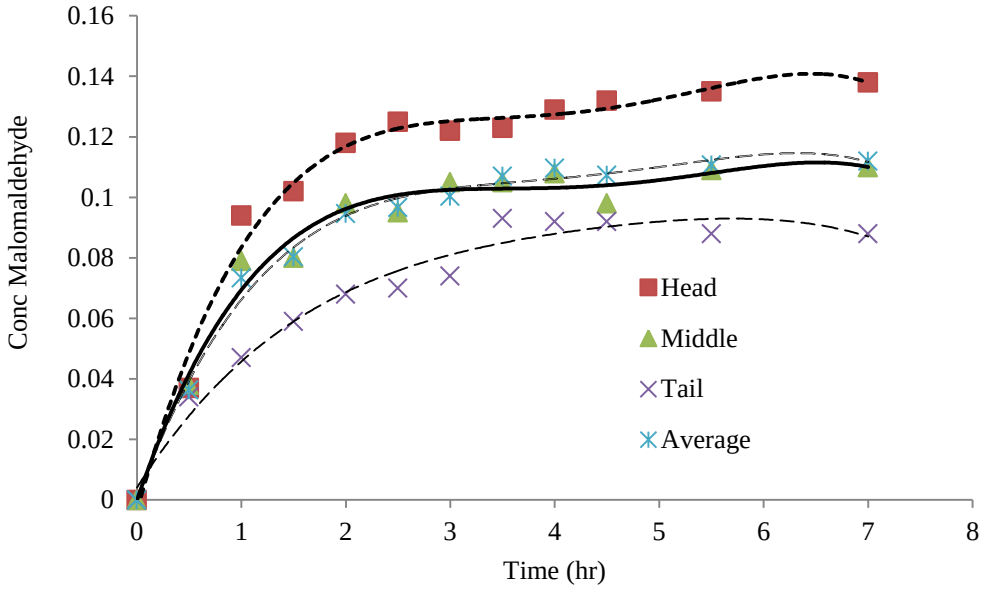


Figure-2: Mean of concentration of Malonaldehyde in samples from different sections of the fish, with time for different anti-oxidant (Triplicate).

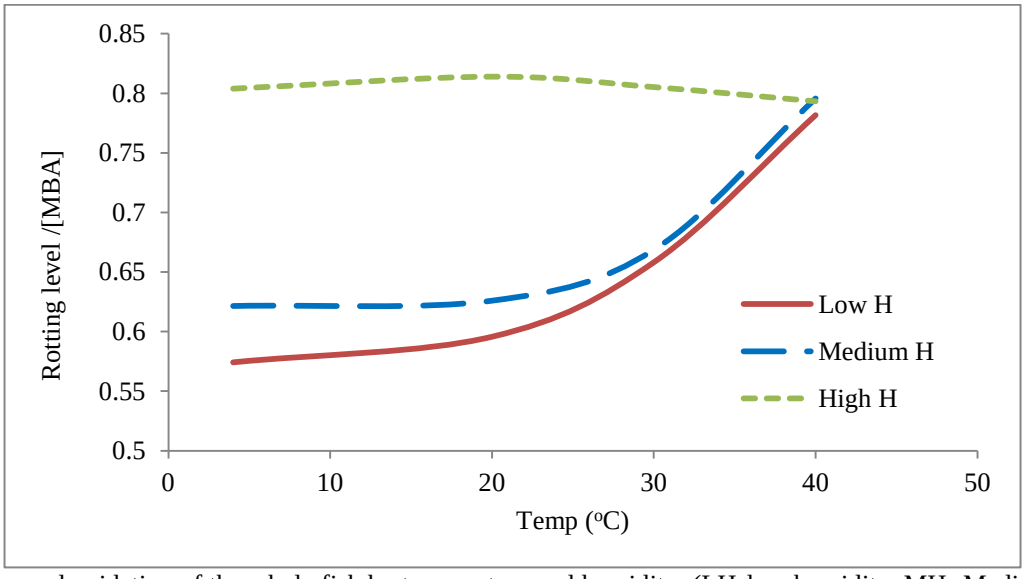


Figure-3: The averaged oxidation of the whole fish by temperature and humidity: (LH-low humidity, MH- Medium humidity, HH-High humidity).

Table-2: The coefficient of determination (R^2) showing the effects of humidity on oxidation extent by body parts as a factor of temperature (from 4.0 to 40.0 ^oC)(Values ± 0.01).

Humidity	Head	Middle	Tail
Low	0.91	0.82	0.65
Medium	0.90	0.79	0.64
High	0.29	0.02	0.18

From Table-2, the tail region of the fish was moderately (≈ 65.0 %) dependent at low to medium humidity levels. The middle part has higher reliance on the humidity at ≈ 80.0 % for the low to medium conditions. However, the head portion of the fish gets more humidity dependent rotting at the low to medium levels with temperature rise. This could be as result of the head being highly contaminated with microbes through the gills (filter feeder), the eyes and mouth parts that enhance deterioration of the condition of the fish by triggering metabolic process through the GLUT 4 pathway eventually leading to increase in Malonaldehyde in fish tissue thus enhancing rotting.

In conformity to Figure-3, the variation in temperature did not affect the rotting of any fish parts at high humidity. These findings agree with Brigitte et al.[25] who observed that watery products at 25^oC will spoil much quickly than a dry, acidic product at 5^oC. Antolovich et al.[3] surmised that considerable amount of evidence is accumulating to suggest that synergism between aqueous and lipophilic systems is the important factor. Thus it is evident that lowering the refrigeration or storage temperatures (10°C) would be beneficial to the head of the fish but only at low to moderately humid conditions. From the above findings and to safe on energy, it is advisable to separate the head from the rest of the body so as to offer each part of the fish its required specific refrigeration conditions.

Effects of different amounts of Anti-oxidants were also considered after three hours of exposure. The effectiveness of different anti-oxidants is displayed in Figure-5.

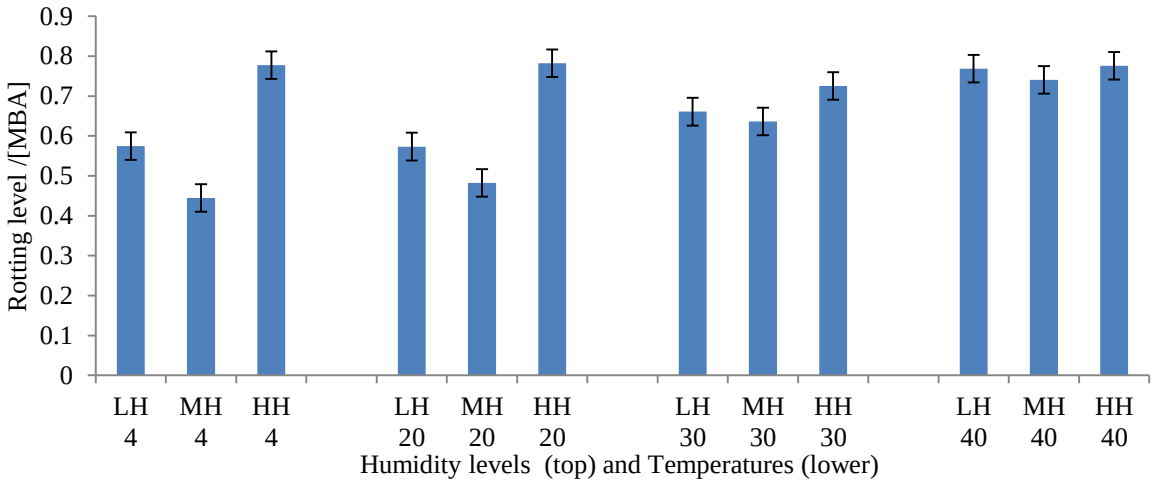


Figure-4: Overall oxidation levels for whole fish with temperature and humidity. (LH= Low humidity, MH = Medium humidity and HH=High humidity).

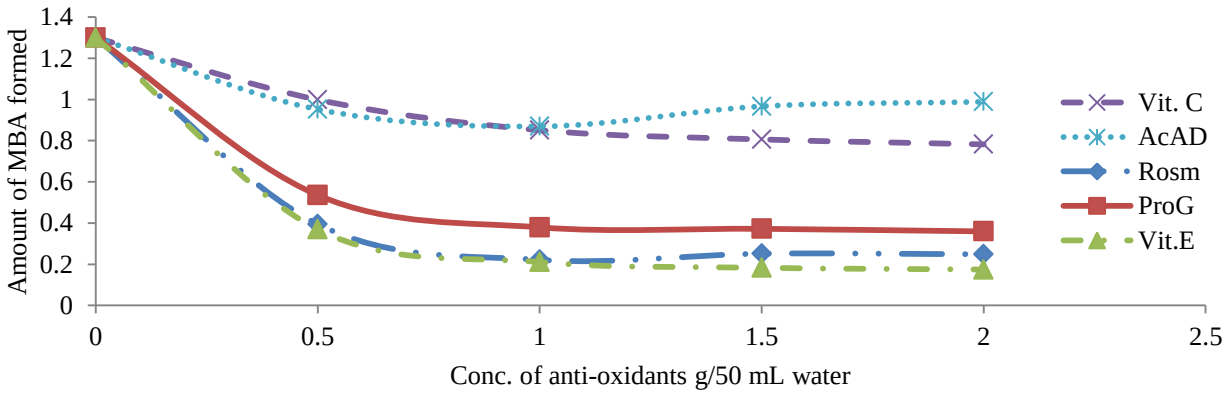


Figure-5: Effectiveness of different concentrations of anti-oxidants in reducing rotting in fish as indicated by amounts of MBA formed.

The relative efficiencies of the anti-oxidants were in the order: Vitamin E ≥ Rosmarinic acid > Propyl gallate > Acetic acid >Vitamin Cat all concentrations. A germain attempt to rank the anti-oxidants was reported by Rezaeizadeh et al.[29] who also observed that Vitamin E performed better as an anti-oxidant in a diabetic rat than Vitamin C. Vitamin E inhibits lipid peroxidation by donation of its phenolic hydrogen to the peroxyl radicals forming tocopheroxyl radicals that, despite being radicals, are unreactive therefore cannotpromote the oxidative chain reaction. Vitamin E is the only major lipid-soluble, chain breaking antioxidant present in plasma, red cells and tissues, imbuing it with capacity to protect the integrity of lipid structures, mainly membrane[3,30].

According to Antolovich et al.[3], interpreting activity of anti-oxidants need careful analysis since their actions may be by different means such as mechanistic intervention, *e.g.*, free radical scavenger, catalytic decomposition, pro-oxidant suppression; rate of scavenging, *e.g.*, near-diffusion or controlled; medium or substrate selectivity (*e.g.*, aqueous, surface or lipid phase); concentration effectiveness (moles of free radicals scavenged per mole of antioxidant); synergistic effect for other antioxidants. None the less, from Figure-5., the concentration at 1g/50mL water of anti-oxidant seems to be the most effective amount to apply. However, even though the efficacy of almost all the anti-oxidants continues to increase beyond 1g/50mL of water, the increase is minimal. In a phenomenon hitherto not reported previously, the efficacy of acetic acid hits a maximum at 1g/50mL of water then decreases at higher concentrations. In the chemical kinetics, such a scenario is consistent with unequal energetics for surface adsorbent-adsorbate interactions. This implies that as more of anti-oxidants are added, equal access to the substrate surface is hindered by the anti-oxidants accumulating in non-single layer formations[31].

To conceptualize the long term impacts of the anti-oxidants, the spoilage of fish when treated with the Rosmarinic acid and Propyl gallate was monitored with time and the trend plotted in Figure-6. The results show that even though effectiveness of propyl gallate as compared to that of Rosmarinic acid increases with time, the effectiveness of Rosmarinic acid peaks at 20 hours then levels off while that of propyl gallate continues to protect more fish tissue.

An important aspect is that TBA does not just measure the oxidation process but also other secondary products of spoilage, and this is why it is also referred to as thiobarbituric acid reactive substances (TBARS). An important difference exists between short- and long-term antioxidant protection. This is correlated to the reaction kinetics and the rate at which an antioxidant reacts with a specific radical *versus* the thermodynamics of the reaction and the extent to which the antioxidant reacts[3].

For ease, antioxidants have generally classified into two groups, namely; primary (chain breaking) antioxidants and secondary (preventative) antioxidants. Secondary antioxidants are compounds that inhibit the rate of oxidation either by removal of substrate or singlet oxygen quenching. Primary antioxidants on the other hand, either inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals. Thus we may predict the anti-oxidative activities by the analysis of these chemical and physical properties.

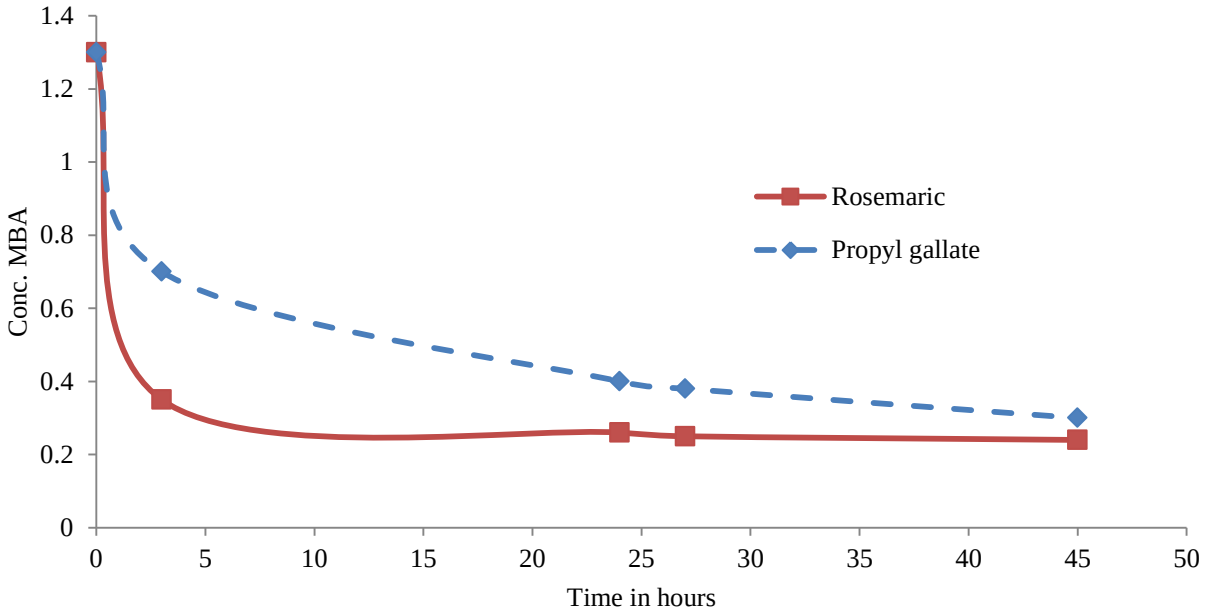


Figure-6: Efficacy of Propyl gallate and Rosmarinic acid with time. (Triplicate).

Table-3: The R^2 for effects of different properties of the anti-oxidants.

Item	Lowest pK_a (Strong acids)	Rotatable bond counts	Kinetic diameter (Å)	Hydrogen count (acceptor)	Polar Surface Area $Å^2$
Logarithmic (R^2)	0.28	0.68	0.81	0.11	0.33

Table-3 shows that pK_a (acidity of the molecule), the hydrogen accepting property of the molecule and the polar surface area did not contribute to the effectiveness of the anti-oxidant molecules as they followed no logical order (Table-1). However, the rotatable bond counts and the kinetic diameters of the anti-oxidants to a larger extend of61% and 81% respectively determined the efficacies of the anti-oxidants. This finding is consistent with the anti-oxidants acting more like a cover-mat in blocking the tissue spoilage. The fact that hydrogen accepting ability showed little influence is highly indicative of preventive anti-oxidant class of preservatives. The property that a compound should have to be considered an antioxidant is the ability of scavenging the radical to form a new radical that is stable through intra-molecular hydrogen bonding[30] which is not observed in this case. Thus, it may appear that for fish tissues, most effective preservatives are those with high rotatable bond counts and larger kinetic diameters as observed in Table-1.

Conclusion

This investigation revealed that there was a high interaction between humidity, temperature and time on oxidation process of fish tissues. In addition, the body parts of the fish were impacted differently by these three environmental parameters. The head is more susceptible to lipid oxidation, followed by the middle section and lastly the tail region. Since the part leading in amount of oxidation products is the head and therefore, for a whole fish, it may be used to represent the overall oxidation levels for the fish sample. Thus it should be advised that for better results on aerobically refrigerated fish, lower ambient humidity is desired and that the head needs to be removed from the rest of the body. For the first time an attempt is made to relate chemical evaluation in terms of MBA value and averaged organoleptic detection, namely, smell. An MBA level of 0.13a.u., which was detected at the 6^{th} hour was the minimum smell detectable value for the rotting fish. The relative efficiencies of the anti-oxidants are in the order of Vitamin E ≥ Rosmarinic acid > Propyl gallate > Citric acid >Vitamin Cat all concentrations. Clearly, for fish tissues, the most effective preservatives are those with high rotatable chemical bond counts and larger kinetic diameters.

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