THE INTERNATIONAL JOURNAL OF
SCIENCE & TECHNOLEDGE

The Effect of Ascorbic Acid and Citric Acid Solution Treatment on the Flesh Quality of *Wallago attu*

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Abstract:
Cat fish Wallago attu was treated with 0.5% Ascorbic and Citric acid solution and kept in frozen storage-12±2°C for 30 days. Changes in proximate composition (protein, lipid, moisture and ash) and biochemical parameters viz. extract release volume (ERV), free fatty acids(FFA) and thiobarbituric acid (TBA) were analyzed and compared with the untreated control samples. Significant increase in FFA and TBA profiles was observed in untreated control samples at the end of storage period. However, the increase was very low in ascorbic and citric acid treated samples. It is due to the anti-oxidative nature of ascorbic and citric acids.

Keywords*: Wallago attu, ascorbic acid, citric acid, free fatty acid and thiobarbituric acid*

1. Introduction

Fish are a perfect food, particularly in terms of protein, vitamin D and trace elements (certain elements found in minimal quantities in the body but which are still of great importance to it). Fish encourages growth and enables tissues to recover because of minerals such as phosphorus, sulphur and vanadium. Fish meat also assists in the formation of healthy teeth and gums, benefits the complexion, makes the hair healthier and contributes to the fight against bacterial infection. Fishes are source of good quality protein that we can easily and completely digest In addition, modern science has also discovered that the omega-3 fatty acids in fish also occupy an important place in human health. These fats have even been described as essential fatty acids. Types 2 diabetes can be prevented by eating fish at least twice a week. Omega 3 fatty acid in fish helps lower the level of triglycerides and increase HDL which is good cholesterol, thus reducing the risk of heart disease. Fish is one of the most perishable food and is very susceptible to microbial and chemical deterioration like lipid oxidation, hydrolysis and protein denaturation etc. Different spoilage mechanisms reported to be involved in this quality loss include microbial development, endogenous enzyme activity, non enzymatic lipid oxidation and enzymatic browning (Ozogul etal, 2006, Auborg, 2004) thus, decreasing the shelf life of fish. Contamination with spoilage microorganisms is almost unavoidable because fish is a very good culture media. During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat. This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids. Higher energy demanding freeze-storage preservation can be altered by synthetic or natural preservatives for control of lipid oxidation and microbial growth in fish during storage (Mahmoud et al., 2006). Combination of these preservatives and refrigeration diminishes the process of spoilage (Bagamboula et al., 2004). Antioxidants are important both from the perspective of food products and for their implication on human health. In food, antioxidants are added to improve the quality and sensory attributes such as color, flavor and texture. In human nutrition, antioxidants play an important role in promoting health and in preventing disease (Raghavan et al., 2010). One preventive measure of oxidative rancidity is the use of single or combined antioxidant treatments. Ascorbic acid and citric acid and their salts are widely known for their role as chelators, acidulates in biological system and synergists of primary antioxidants, so that a profitable effect on fish oil and emulsions (Kelleher et al, 1992; Osborn-Barnes and Akoh, 2003), minced fish (Stodolnik et al, 1992) and fish fillets (Badii and Howell, 2002; Auborg et al, 2004) have been observed. Hence, this work is aimed to study the effect of Ascorbic acid and Citric acid on extension of shelf life of cat fish *Wallago attu.*

2. Materials and Methods

2.1. Collection of fish samples

Fresh samples of cat fish *Wallago attu* were purchased from local market of Jammu city. They were immediately brought to the lab in polythene bags along with crushed ice. The viscera of fish were removed and the fish was washed with large amount of water. Analytical procedures for biochemical and microbiological changes were done on 0, 10th, 20th and 30th day of storage.

2.2. Fish Treatment

The fish was cut in to pieces and these pieces were divided into two groups viz. Gp.A and Gp.B. Gp.A samples were considered as fresh (Control), stored at-12±2°C wrapped in aluminum foil, kept in air tight plastic container without pre treatment of 0.5% Ascorbic acid and Citric acid solution while the second group, Gp.B samples were dipped in the solution of 0.5% Ascorbic acid and Citric acid for 15 minutes, taken out and immediately wrapped in aluminum foil, kept in air tight plastic container and stored at-12±2°C (frozen storage).

2.3. Analyses

The proximate composition (ash and moisture) of the fish samples were evaluated using the standard AOAC procedure (AOAC, 1995). The protein content was determined using the Lowry *et al.* (1951). Fat content was determined using Folch *et al* (1957). Thiobarbituric acid value of fish muscle during storage was determined using the method of Witte *et al* (1970). Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick) described by Koniecko (1979). Extract Release Volume (ERV) was determined as per the method of Strange *et al.* (1977). The pH of fish muscles was determined by the method of Keller *et al.* (1974). The microbiological profile was determined according to APHA method (1984). Data were expressed as mean ± SD and were analyzed by one-way ANOVA test using SPSS statistical programme.

2.4. Statistical Analysis

Means and standard errors were calculated for different parameters. The data analyses were performed using SPSS software (12.0 for Windows). Differences between treatments were analyzed using independent-measures one-way ANOVA. Post-hoc comparisons were conducted using Duncan's test. The values were expressed as mean ± SE. values <0.05 were considered as significant and p values <0.001were considered as highly significant *p*.

3. Results and Discussion

3.1. Proximate Composition

Triplicate flesh samples of *Wallago attu* with and without the treatment of (A.A& C.A) were analyzed for determining its proximate composition viz. protein, lipid, moisture and ash content during 30 days of frozen storage period.

3.1.1. Protein content

In the present study, the protein content of frozen muscle sample of untreated control Gp. A was 15.45% on day 0 and 10.14% on day 30th. Further, a significant ($P \leq 0.05$) percental decrease was found in total protein content i.e 8.67%,20.71% and 34.3% on 10th, 20th and 30th day respectively. These results are in line with Arannilewa *et al* (2005) in Tilapia fish (*Sarotherodun galiaenus*), Siddique *et al* (2011) in *Puntius* and Gandotra *et al* (2012) in *Labeo rohita*, who suggested that loss of protein might be due to leaching effect of amino acids with melting ice.

Further, muscle samples of treated Gp. B i.e A.A and C.A treated samples showed a comparatively less percental decrease in protein content i.e. 5.73%, 11.40% and 20.11% on 10th, 20th and 30th day respectively. These findings are supported by the studies of Omojowo *et al* (2005) in Cat fish (*Clarius garipinus*), Nessrien *et al* (2007) in Mullet fish muscle and Arekemase *et al* (2012) in Tilapia and Mackerel who opined that the antioxidants have the ability to slow down the protein autolytic process in muscle which resulted in delayed muscle break down.

DAYS	PROTEIN	LIPID	ASH	MOISTURE
0 day	15.45±0.03	4.02±0.02	1.03±0.3	81.66±0.06
10th day	14.11±0.5	3.44±0.02	0.98±0.05	79.20±0.2
20th day	12.25±0.1	3.00±0.01	0.94±0.05	76.94±0.11
30th day	10.14±0.02	2.36±0.1	0.92±0.03	74.00±0.1

Table 1-Proximate composition (wet weight basis) of raw fish muscle of Wallago attu stored in freezer at -12±2ºC for a period of 30 days.(Gp.A)

DAYS	PROTEIN%	LIPID%	ASH%	MOISTURE%
0-10	8.67%	14.42%	4.8%	3.01%
0-20	20.71%	25.37%	8.7%	5.78%
0-30	34.3%	41.29%	10.67%	9.3%

Table 1(A)Percent decrease in proximate composition of raw fish muscle of Wallago attu stored in freezer at -12±2ºC for a period of 30 days.(Gp.A)

DAYS	PROTEIN	LIPID	ASH	MOISTURE
0 day	15.24±0.02	4.21±0.04	1.02±0.1	81.67±0.03
10th day	14.34±0.05	3.93±0.01	1.00±0.3	80.42±0.02
20th day	13.41±0.02	3.59±0.05	0.97±0.4	78.71±0.02
30th day	12.08±0.1	3.19±0.05	0.94±0.1	77.67±0.1

Table 2- Proximate composition (wet weight basis) of fish muscle of Wallago attu treated with ascorbic and citric acid.

DAYS	PROTEIN%	LIPID%	ASH%	MOISTURE%
0-10	5.90%	6.65%	1.96%	1.53%
0-20	12.00%	14.72%	4.90%	3.62%
0-30	20.73%	24.22%	7.84%	4.89%

Table 2(A)-Percent decrease in proximate composition of fish muscle of Wallago attu treated with ascorbic and citric acid, stored in freezer at -12±2°C for a period of 30 days.(Gp.B)

3.1.2. Lipid content

A decrease from 4.02% on day 0 to 2.36% on day 30th was observed in the lipid content of raw muscle . Total percental decrease was 14.42%, 25.37% and 41.29% on 10th, 20th and 30th day respectively during frozen storage. Similar observations were made earlier by Zoldos *et al* (2010) in Allaska Pollack, Siddique *et al* (2011) in *Puntius sps.* and Gandotra *et al* (2012) in *Labeo rohita*. They attributed this loss in lipid to the oxidation of lipids.

However, the treated samples (Gp. B) also revealed a decreasing trend in lipid values but the decrease was low when compared to untreated Gp. A. The total percental decrease in Gp.B (treated) was 6.65%, 14.72% and 24.22% on 10th,20th and 30th day of storage respectively. These results are favored by the findings of Jamilah *et al* (2008), Arekemase *et al* (2012), Ehsani and Jasour (2012) and Rahimabadi and Divband (2012) who proposed that highest fat content and low free fatty acids in fish muscle samples treated with antioxidants after a prolonged storage may be due to the prevention of oxidation and hydrolysis of lipids in fish by anti-oxidants during frozen storage.

3.1.3. Moisture content

The total moisture content decreased with the increase in storage time in both Gp. A (untreated) and Gp.B (treated) muscle samples respectively. There was a 3.01%, 6.78% and 9.3% decrease in Gp. A and 1.53%, 3.62% and 4.89% decrease in Gp. B on 10th, 20th and 30th day of storage respectively. These results get support by the findings of Le Blanc and Le Blanc (1992), Bekelvik *et al* (2005) in Sea Bass and Emire *et al* (2009) in Nile Tilapia during frozen storage. They attributed this moisture loss to the condensation of water during chilling.

3.1.4. Ash content

Due to low bone mass in *Wallago attu*, the ash content calculated is very low and it also showed a decreasing trend with increase in storage period in both Gp. A and Gp. B showed a decrease in ash content during the frozen storage conditions. The total percental decrease was 4.8% and 1.96% on 10th day, 8.7% and 4.90% on 20th day and 10.67% and 7.84% in Gp. A and Gp. B respectively. Similar results were obtained by Bekelvik *et al* (2005) in Sea Bass and Emire *et al* (2009) in Nile tilapia and Okeyo *et al* (2009) in frozen Nile Perch. This decrease in ash may be attributed to the drip loss, resulting in loss of bulk and trace elements.

3.2. Chemical Analysis

DAYS	FFA	TBA	ERV
0 day	0.54±0.01	0.10±0.03	26±0.01
10th day	4.82±0.3	2.95±0.05	31±0.5
20th day	9.02±0.04	3.75±0.01	37±0.05
30th day	14.55±0.05	5.45±0.04	44.45±0.02

Table 3- Change in bio-chemical composition of raw fish muscle of Wallago attu stored in freezer at -12±2°C for a period of 30 days.(Gp.A)

DAYS	FFA	TBA	ERV
0 day	0.28±0.01	0.11±0.4	23±0.04
10th day	0.57±0.03	0.44±0.1	23±0.05
20th day	0.93±0.2	0.95±0.1	25±0.5
30th day	1.36±0.02	1.44±0.2	27±0.04

Table 4- Change in bio-chemical composition of fish muscle of Wallago attu treated with ascorbic and citric acid, stored in freezer at -12±2°C for a period of 30 days.(Gp.B)

3.2.1. Extract Release Volume (ERV)

Both Gp. A and Gp. B showed a progressive increase for extract release volume values from 0th to 30th day. In Gp. A, the values were 26±0.01 ml on 0 day and 31±0.5 ml, 37.00±0.05 ml and 44.45±0.02 ml on 10th, 20th and 30th day respectively. However, in Gp. B, the initial value was 23±0.04 and after 30 days, it increased upto 27±0.04.The total percental increase in ERV was very low in Gp.B (treated) i.e. 14.81% as compared to the Gp.A i.e.41.50% through out the storage period. These results are corresponding with the results of Rostamzad *et al* (2011) in Persian Sturgeon, Pourashouri *et al* (2011) in Wels Catfish and Taheri *et al*(2012) in Cobia, who attributed this less increase in ERV to the increased water holding capacity in anti-oxidant (ascorbic acid and citric acid) treated samples. As water holding capacity in meat tissue is strongly related to myofibril protein structure, therefore, this less increase in ERV in present studies may be due to the effect of antioxidants in delaying the protein denaturation.

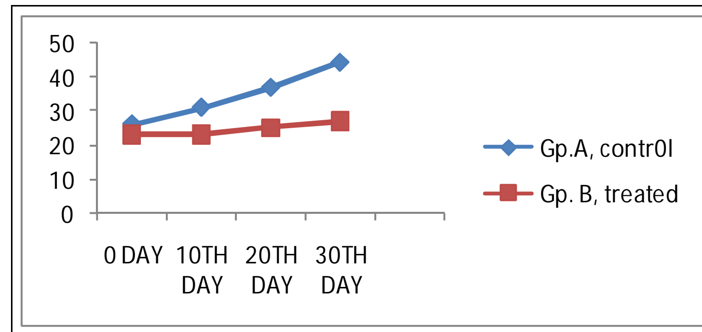

Figure 1: Comparison of values of Extract release volume (ERV) for Gp. A(control) and Gp. B (treated) muscle samples

3.2.2. Free Fatty Acids (FFA)

Free fatty acids result in the production of off-flavour and undesirable taste producing low molecular weight compounds after oxidation. FFA determine the deterioration of fats due to their hydrolysis. Results for both treated and untreated muscle samples depicted an increase in FFA under frozen storage. In Gp. A, FFA values on day 0, 10th, 20th and 30th day were 0.54%, 4.82%, 9.02% and 14.55% respectively. A gradual increase in FFA formation so observed in treated Gp. B samples is shown in Table 4. But the increase in FFA was low in Gp. B samples as compared to Gp. A samples. Similar increasing trend in FFA was found by Stodolnik et al (2005) in Mackerel (*Scomber scombrus*), Ozogul *et al* (2011) in Common sole (*Solea solea*) and Jezek and Buchtova (2012) in freeze thawed fillets of Common carp and Silver carp. Aubourg *et al* (2004), Pourashouri *et al* (2011) in Wels catfish (*Silurus glanis*), Rostamzad *et al*(2011) in Persian sturgeon, Taheri *et al* (2011) and Pieretti *et al* (2012) in Rainbow trout and Nitipong et al (2014) in snake headed fish (*Channa striata*). They associated this increase in FFA to the hydrolysis of fat and oil present in fish muscle. This slow increase in FFA formation in Gp. B is attributed to the fact that Ascorbic and Citric acid act as oxygen scavengers and metal chelators, thus causing delay in lipid oxidation. (Rostamzad, 2011).

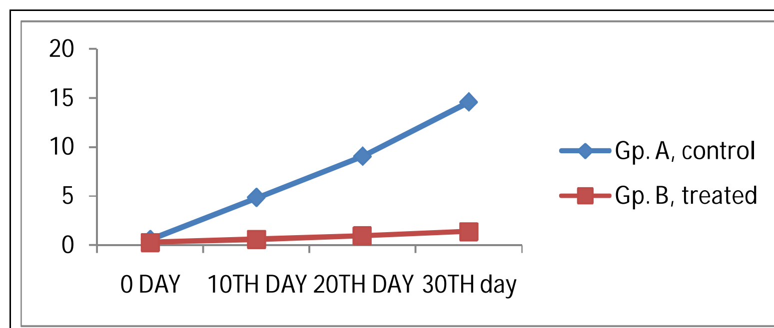

Figure 2: Comparison of the free fatty acid values of Gp. A(control) and Gp. B (treated) muscle samples

3.2.3. Thiobarbituric acid TBA

The TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content and widely used for assessment of degree of lipid oxidation. MDA is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen.(Sallam,2007).

Persuals of table 3 and 4 show an increase in TBA (mg malonaldehyde/ kg) values in both Gp.A and Gp.B samples with increase in storage period. However, the increase was lower in Gp.A (untreated) samples when compared to Gp. B(Treated) samples. Similar results were found by Rostamzad *et al*(2011) in Persian sturgeon, Taheri *et al*(2012) in cobia (*Rechycentron canadum*), Zakipour and Dirband *et al*(2012) in Silver carp, Hassanin and El-Daly (2013) in Nile Tilapia (*Oreochromis niloticus*)and Nitipong *et al* (2014) in *Channa striata.* They suggested that lower TBA values in treated samples was due to the positive effect of anti-oxidants in delaying lipid oxidation.

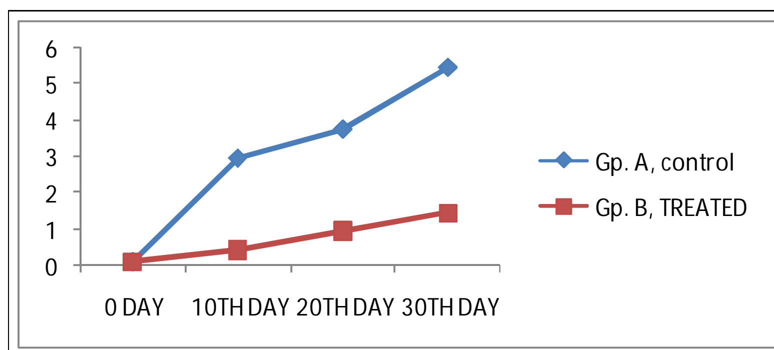

Figure 3: Comparison of the Thiobarbituric acid values of Gp. A (control) and Gp. B (treated) muscle samples

4. Conclusion

The present studies reveal an increase in shelf life of the muscle samples treated with 0.5% Ascorbic and citric(AA+CA) acid due to slow hydrolysis and oxidation of lipids. This is due to the oxygen scavenging effect of ascorbic and citric acids. Thus, keeping in mind easy availability and role in inhibiting the deterioration of fish muscle by 0.5% AA+CA solutions, their use is recommended to enhance the shelf life of fish.

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