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Effects of Hydroalcoholic and Water Extracts of Nettle Leaf (*Urtica dioica L.*) on Chemical Properties of Superchilled Minced Meat of Common Kilka (*Clupeonella cultriventris caspia*)

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Abstract

Background: Antioxidant activities of plant extracts are being studied to increase the shelf life of fish products. Some plants such as *Urtica dioica L.* (nettle) can be a good source of antioxidants. The objective of this study was to evaluate the effects of hydroalcoholic and water extracts of nettle leaf on the chemical properties of superchilled minced meat of the common kilka fish (*Clupeonella cultriventris caspia*).

Methods: One hundred g of minced meat of kilka was mixed with 200 and 400 ppm of both hydroalcoholic and water extracts of nettle separately, and stored at -2 °C. The Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric Acid Reactive Substances (TBARS) of the samples were analyzed at days 0, 3, 7, 14, 21 and 28. The statistical analysis was carried out using Duncan's multiple range tests in MStat-C software.

Results: Both hydroalcoholic and water extracts of nettle extended the shelf life of minced kilka from 2 days to 8 days. However, no relation (p>0.05) was observed between chemical values and kind of extracts. Also, there was no significant difference (p>0.05) between two concentration levels of extracts and chemical values.

Conclusion: Treatment levels of nettle extracts could effectively delay chemical deterioration, and extend the shelf life of minced kilka during storage. A combination of superchilling temperature (-2 °C) and treatment of minced kilka with 200 and 400 ppm of hydroalcoholic and water extracts of nettle can extend the shelf life to 28 days. Based on the results of this study, it is proposed that these plant extracts can be used as safe preservatives for minced fish.

Introduction

The most common form of chemical deterioration of fish and fish products is oxidative rancidity (Kanner, 1994), which can involve extensive flavor changes, color losses and structural damage of proteins (Xiong, 1996). Rancidity of fish products changes their odor, flavor, taste, color, texture and appearance. Also, these changes involve free-radical production which can cause adverse health effects. Therefore, delaying lipid oxidation and product enhancement are factors that can contribute to the development of functional meat and fishery products with enhanced nutritional and health benefits, improved shelf-life and superior product quality (Hayes et al., 2011).

Several synthetic antioxidants have been used to prevent lipid oxidation in meats. The addition of various synthetic antioxidants such as Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and Tertiary Butyl

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Hydro Quinone (TBHQ) is reported to be very effective (Khalil and Mansour, 1998). Since the use of these synthetic antioxidants has been restricted because of their possible health risks and toxicity (Buxiang and Fukuhara, 1997) and consumers demand for additive-free or natural products (Ahn et al., 2002), the development of effective natural antioxidants have been investigated in meat products (Atrea et al., 2009).

Plant extracts with antioxidant and antimicrobial activities are being studied to prolong the shelf life of meat, fish, poultry, and their products. Some plants such as *Urtica dioica L.* (nettle) that are abundant in the north of Iran can be a good source of antioxidants. A nettle extract prevent free radical formation in lipid oxidation (Exarchou et al., 2006) and its leaves are a good source of essential amino acids, ascorbic acid and other minerals, especially iron (Martinez et al., 2006). Dried-granulated nettle leaves have antioxidant and antimicrobial (Aksu and Kaya, 2004) effects on fermented meat products. It was reported that a nettle water extract has more antioxidant activity than BHA, quercetin, and α-tocoferrol, and also has antimicrobial effects on various bacteria in vitro (Alp and Aksu, 2010). Also, nettle is heavily used in the medical sector due to its antiviral properties (Manganelli et al., 2005).

To our knowledge, there are no studies in the literature on common kilka meat treated with nettle extracts. The objective of this study was to determine the effects of hydroalcoholic and water extracts of nettle leaf (*Urtica dioica L.*) on chemical properties of superchilled minced meat of common kilka (*Clupeonella cultriventris caspia*).

Materials and methods

Preparation of hydroalcoholic extraction of nettle leaves

Aerial parts of nettle were collected in April 2012 from Mazandaran, Iran and identified in the Biology Department at Mazandaran University, Iran. The aerial part of the plant was washed and dried at 55 °C in the oven. For hydroalcoholic extraction, dried aerial parts of nettle were ground to a fine powder in a mill. Then, 10 g of fine powder was mixed with 100 ml ethanol at room temperature in a shaker. The extraction was carried out in the dark. After filtering the obtained extract with clean cotton, the remained materials were extracted as mentioned before for second time. Then, the hydroalcoholic extracts were mixed. After evaporating the ethanol at <40 °C in the rotary evaporator, the hydroalcoholic extract of nettle was placed in a plastic bottle, and then stored at -20 °C until used (Gulcin et al., 2004).

Water extraction preparation of nettle leaves

Nettle samples were left on a bench to dry. The dried sample was chopped into small parts with a blender. For water extraction, 20 g dried aerial parts of nettle was ground into a fine powder in a mill and was mixed with 400 ml boiling water by magnetic stirrer for 15 min. Then, the extract was filtered through Whatman no. 1 paper.

The water extract of nettle was placed in a plastic bottle, and then stored at -20 °C until next step of the experiment (Gulcin et al., 2004).

Fish preparation

Fifty live commercial-sized kilka with an average weight of 7 g were used in this study. They were caught in the Anzali area of Caspian Sea in March 2013 and, transferred to the Food Processing Laboratory of National Fish Processing Research Center, Bandar Anzali, Gilan, Iran, within 1 h and kept alive before being processed. The fishes were killed by slurry ice and kept whole at 0 °C before use (Li et al., 2012).

Preparation of fish samples

The fish were washed, gutted, skinned and minced, respectively. Then, 100 g of minced meat was mixed with 200 and 400 ppm of both hydroalcoholic and water extracts of nettle, stored at -2 °C as superchilling temperature for seafood storage (Erikson et al., 2011). Sampling was done after 0, 3, 7, 14 and 28 days after preparation and tested for chemical properties.

Determination of Total Volatile Basic Nitrogen

Total Volatile Basic Nitrogen (TVB-N) value was estimated as described by FOSS (2002). The micro diffusion method was menstruated by distillation after adding magnesium oxide to the homogenized samples. TVB-N values were determined with a Kjeltec 2300. TVB-N values were expressed in mg N/100 g fish sample.

Determination of Thiobarbituric Acid Reactive Substances

Thiobarbituric Acid Reactive Substances (TBARS) were determined colorimetrically by the method described by Kirk and Sawyer (1991) as a Pearson modified method. A portion (20 g) of sample was mixed with 50 ml of 20% trichloroacetic acid for 2 min and then was washed with distilled water. The mixture was filtered through Whatman no. 1 paper. In the next step, 5 ml of the obtained extract was mixed with 5 ml of TBA reagent. Absorbance was measured at 532 nm against a water blank. TBA value was expressed as mg of malondialdehyde (MDA) equivalents/kg of fish meat.

Statistical analysis

All experiments were carried out in duplicate. ANOVA and Duncan's multiple range tests were applied to perform

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statistical analysis of data, using MStat-C software. Differences between means were considered statistically significant at the 95% confidence level (p<0.05).

Results

TVB-N and TBARS values were determined to analysis of the chemical quality changes in minced meat of kilka containing different concentrations of nettle leaves water and hydroalcoholic extracts during 28 days storage period. TVB-N and TBARS values were gradually increased in all samples during 28 days storage.

Both hydroalcoholic and water extracts of nettle extended significantly the shelf life of minced kilka from 2 days to 8 days (Fig. 1 and Fig. 2). However, no relation (p>0.05) was observed between chemical values and kind of extracts. Also, there was no significant difference (p>0.05) between two concentration levels of extracts and chemical values.

Discussion

The TVB-N value is one of the most widely used indicators of seafood deterioration. It is a general term which includes the calculation of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during chilled preservation), ammonia (produced by the deamination of amino acids and nucleotide catabolites) as well as other volatile basic nitrogenous compounds correlated with seafood spoilage (Li et al., 2012). TVB-N safety level for human consumption in seafood is under 20 mg N/100 g product.

Our Results showed that the TVB-N values increased gradually in all samples during 28 days storage, which is in agreement with results of Li et al., (2012), about the Crucian carp (*Carassius carassius*) containing natural preservatives. As shown in Fig. 1, the control samples had the highest TVB-N during the storage.

We found that no relation (p>0.05) was between chemical values and kind of extracts. These results verified the study of Arashisar et al., (2008) about TBARS in rainbow trout fillets. The safe level of TBARS in seafood for human consumption is under 3 mg MDA/kg.

Based on the results of this study, there was no statistically significant difference (p>0.05) between two concentration levels of extracts and chemical values that is in accordance with the study of Arashisar et al. (2008) on rainbow trout fillets.

Conclusion

This is the first study of the effects of water and hydroalcoholic extracts of *Urtica dioica L.* on the shelf life of superchilled minced kilka. It is concluded that natural nettle extracts can be used as an effective preservative for the storage of minced kilka fish products.

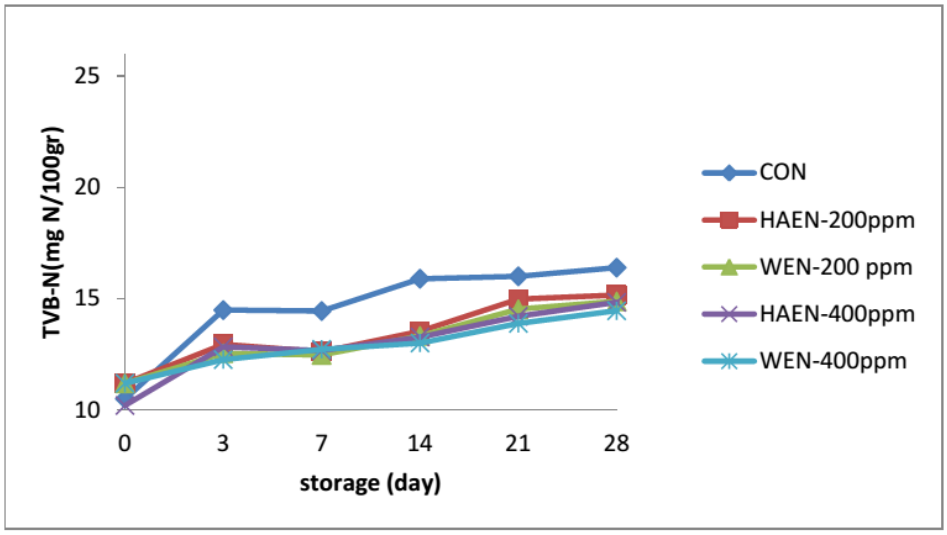


Fig. 1: Changes in TVB-N values of minced kilka samples treated with Hydroalcoholic Extract of Nettle (HAEN), Water Extract of Nettle (WEN) and Control (CON) during storage period at -2 ℃

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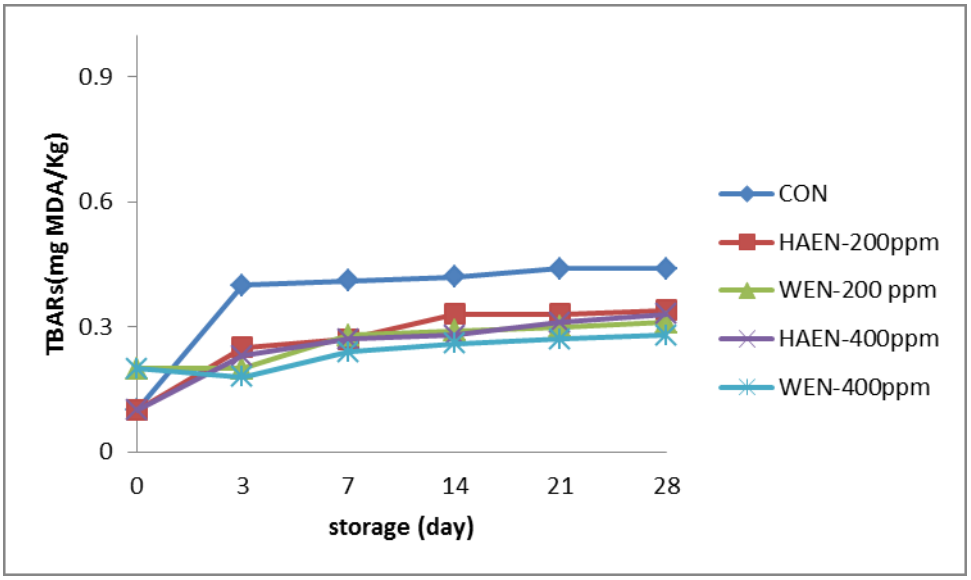


Fig. 2: Changes in TBARS values of minced kilka samples treated with Hydroalcoholic Extract of Nettle (HAEN), Water Extract of Nettle (WEN) and Control (CON) during storage period at -2 °C

Conflicts of interest

The authors declare that they have no conflict of interest.

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