Effects of *Mentha pulegium* water extract dipping on quality and shelf life of silver carp (*Hypophthalmichthys molitrix*) during superchilled storage

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Abstract

The effects of *Mentha pulegium* water extract dipping on quality and shelf life of silver carp during superchilled storage were investigated. Fish samples were treated with water extract of 1 and 3%*M.pulegium*, and then stored at -3 ^{o}C for 30 days. The control and the treatedfish samples were analyzed periodically for chemical (pH, PV, TBA, TVB-N), and sensory characteristics. The results indicated that the effect of *M.pulegium* extract dipping on fish samples was to retain their good quality characteristics and extend the shelf life during superchilled storage, which was supported by the results of chemical and sensory evaluation analyses. Inthis respect, the sample supplemented with 3% water extract was more potent compared with the 1% one in extending the shelf life of fish fillets.

Keywords: Silver carp, *Mentha pulegium*, Water extract, Superchilled storage, Quality.

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Introduction

Since variety of fishes as an animal protein source has a high nutritive value, so many countries have tried to increase per capita consumption of this nutritive source (Khoshkhoo et al., 2012). Carp is one of the most widely cultured and traded species all over the world (Fan et al., 2009). Silver carp (*Hypophthalmichthys molitrix*) is the most important species in the poly-culture system in Iran and its aquaculture production has rapidly increased during last years (Aquaculture Department., 2003;Zakipour Rahimabadi and Divband., 2012). However, fish are more susceptible than other muscle foods to both microbial and chemical deterioration due to the abundance of polyunsaturated fatty acid and protein present in their flesh (Vidya Sagar Reddy and Srikar., 1996).

Frozen storage is a general preservation method, used to control or decrease biochemical changes in fish that occur during storage. Since freezing modifies the structure of the organoleptic properties of the product, storing food just above the initial freezing temperature (e.g. at -2 /-3 °C for fish meat) or superchilling has recently been considered as an innovative technology for retarding spoilage of fish meat. Nevertheless, this method does not completely inhibit microbial and chemical reactions that lead to quality deterioration of fish due to its muscle chemical composition (Vidya Sagar Reddy and Srikar, 1996; Beaufort et al., 2009).

To retain the good quality characteristics for longer, and extend shelf life during frozen storage of fish, chemical preservatives, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been widely used. At the same time, in order to avoid the use of synthetic preservatives, which do more harm than good, numerous studies are currently focused on using natural ingredients to enhance fish quality and shelf life (Fan et al., 2009; Khanedan et al., 2011); moreover, it has been shown that the incorporation of the antioxidants BHA (butylated hydroxy anisol), propyl gallate, and/or citric acid into meat prior to freezing, considerably inhibited lipid oxidation, but did not effectively protect against the functional deterioration of the flesh- contained protein during short-term storage (Smith., 1987).

The Labiatae family includes about 220 genera and 3300 species which are widely used for various purposes (Evans., 1996). Plants belonging to the Labiatae family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant and antimicrobial properties (Ozgen et al., 2006; Tepe et al., 2006; Mahboubi and Haghi., 2008; Kamkar et al., 2010). The Mentha genus is a member of this family and represents by about 6 species in the flora of Iran (Mozaffarian., 1996). Mentha is generally known under the name *''na'na"* and ''*pooneh"* in Iran and commonly used as herbal tea, flavoring agent, and medicinal plant (Nickavar et al., 2008). However, research on the retention of the good quality characteristics for long periods and the extension of shell life during superchilled storage of fish by Mentha genus species dipping is still lacking.

By considering the mentioned characteristics, the aim of the present study

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was to evaluate the effects of dipping into *Mentha pulegium* water extract on quality and shelf life of silver carp fillet during superchilled storage.

Materials and methods:

Aerial parts of *M. pulegium* L. were collected from Guilan province (Iran) During the flowering period in spring 2011. A voucher specimen for this plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University, Tehran, Iran. The plants were dried in a dark place at room temperature. Dried leaves were powdered using an electric device and stored at refrigerator (4 ^{o}C) until use. Water extract was prepared by means of a soxhlet. In this regard, leaf samples were extracted with distilled water in a soxhlet apparatus until extraction water became colorless (Kamkar et al., 2010).

Phytochemical screening was carried out on the extract using thin layer chromatography (TLC). The extracts were subjected to TLC examination for group determination of the secondary materials. In this respect, Modified Dragendroff's reagent , according to the method of Szymanski et al. (2002) for alkaloids, ferric chloride reagent (07875, Sigma) for phenolics, Naturstoff reagent ,according to the method of Schnitzler et al. (2007) for flavonoids and vanilline/sulfuric acid reagent (Fried and Sherma., 1996) for terpenoids were used. Solvent systems for developing of ready coated analytical TLC plates (Merck Silica gel 60 F254, 0.25 mm, Merck Company supplier, Tehran, Iran) were selected according to the method of Wagner and Bladt (Wagner and Bladt., 1996).

Fresh, live silver carp (*H. molitrix*), in 250 ± 30g weight, were purchased from a local fish market and delivered to the laboratory of Food Hygiene within 12 h, packed in insulated polystyrene boxes, containing flaked ice. The fish were immediately headed, gutted and filleted in order to remove all bones, skin and visible dark meat, and was cut into a 0.5× 3×4 cm sized fillet. After washing, fish samples were given a dip treatment in 1% and 3% *M. pulegium* extract solution (treatment groups) and in distilled water as a control, respectively for 120 min and then well drained. After that, they were individually packed in plastic trays and airproofed with polyvinyl dichloride (PVDC); then all the packs were kept in a refrigerator maintained at -3 °C for 30 days(Duun and Rustad., 2008; Fan et al., 2009) . Fish samples were taken randomly every 5 days for chemical and sensory evaluation.

A 10 g sample of the fish muscle was homogenized in100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a digital pH meter.

50 g of fish fillets were homogenised with 150 ml of chloroform/methanol (2/1:v/v) solution (Merck) using a blender mixer (Waring, USA) (2 × 30 s). The mixture was filtered then added to 50 ml KCl (Merck) solution (0.88%). After decantation, the organic phase (lower phase) was collected and reextracted twice with 100 ml methanol/KCl 0.88% (1/1:v/v) solution. The solvent was made to evaporate using a rotary evaporator at 35°C and the residual solvent was

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removed by flushing with nitrogen. Finally, the obtained oil was used for PV analysis (Zouari et al., 2010).

For PV measurement, lipid sample (1.0 g) was treated with 25 ml of organic solvent mixture (chloroform/acetic acid, 2/3: v/v). The mixture was shaken vigorously, followed by addition of 1 ml of saturated potassium iodide (Merck) solution. The mixture was kept in the dark for 5 min before adding 75 ml of distilled water. 0.5 ml of starch solution (1%, w/v) was added to the mixture, as an indicator. The PV was determined by titrating the iodine liberated from potassium iodide with standardised 0.01 N sodium thiosulfate solution and was expressed as milliequivalents (meq) of peroxide per kg of lipid (Zouari et al., 2010).

TBARS, an index of lipid peroxidation, was determined using the method of Botsoglou et al. (1994), with some modifications. In brief, 1 g of sample was homogenized in the presence of 8 mL of 5 g/100 mL aqueous Trichloroacetic acid (Merck, Darmstadt, Germany) and 5 mL of 0.8 g/ 100 mL BHT in hexane (Merck), and the mixture was centrifuged. The top layer was discarded, and 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL of 0.8 g/ 100 mL aqueous 2-thiobarbituric acid (Merck) to be further incubated at 70^{o}C for 30 min. Following incubation, the mixture was cooled under tap water and its absorbance was measured at 532 nm. Concentrations of TBARS were determined by the standard curve using 1,1,3,3-tetraethoxypropane (Merck, Hohenbrunn, Germany) as the standard (Jebelli Javan et al., 2012).

Total volatile basic nitrogen (TVBN) was determined by distillation afterthe addition of MgO to minced fish sample. The distillate was collected in a flask containing a 3% aqueous solution of boric acid and a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.05 g of methylene blue to 100 ml of ethanol. Finally, the boric acid solution was titrated with a 0.1 N hydrochloric acid solution. Analysis was carried out according to the method described by Pearson (1976) after appropriate modification (Goulas and Kontominas., 2005).

The sensory quality of fish sample was evaluated by a seven member trained panel from the laboratory staff (Fan et al., 2009). Panellists scored for sensory characteristics, such as colour, odour, general acceptability and texture, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely).

Experiments were replicated twice on different occasions with different fish samples. All analyses were run in triplicate for each replicate ($n = 2 \times 3$). All data were subjected to analysis of variance (ANOVA) using the sas software (SAS 1997). The least significant difference (LSD) procedure was used to test for difference between means (significance at $p<.05$).

Results:

Phytochemical study showed that Flavonoid, terpenoid and phenolic compounds were major components of the *M. pulegium* water extract.Variations in values of pH during storage are depicted in Figure 1. The initial pH of the fish sample was found to be 6.6 in all fish samples. pH values in all samples showed a

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trend to increase from beginning of the storage

that a initial decrease of pH values can be seen. For the control sample the gradual increase in pHs from day 15 to end of the storage period was significantly ($p<.05$) higher than the 3% period to the end of experiment except day 5th treated samples but there was no difference between control and 1% treated samples throughout the entire storage period ($p>.05$).

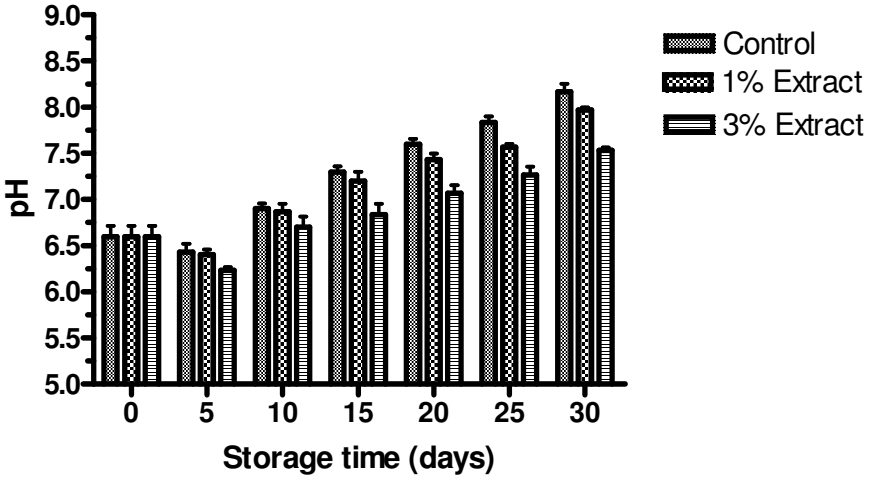


Figure 1: Changes in pH of fish sample during the storage

Figures 2 and 3 show the PVs and TBARS levels during the storage of fish fillets over a 30-day period in the presence of two various concentrations (1 and 3%) of *M. pulegium* water extract. The initial PVs and TBARS levels in the fresh fillets were 2.47 ± 0.15 meq/kg and 0.54 ± 0.02 mg MDA/kg, respectively.

We have shown that *M. pulegium* extract reduced PVs in 3% concentration At different incubation time points compared to the control ($p<.001$).

However, in all samples PVs showed a trend to increase from beginning of the storage period to the end of experiment. For the control and 1% treated sample, the gradual increase in PVs from day 5 to end of the storage period was significantly ($p<.001$) higher than the 3% treated sample. In this respect, we have found that PVs of Control and 1% treated sample during beginning until the end of storage period were similar ($p>.05$).

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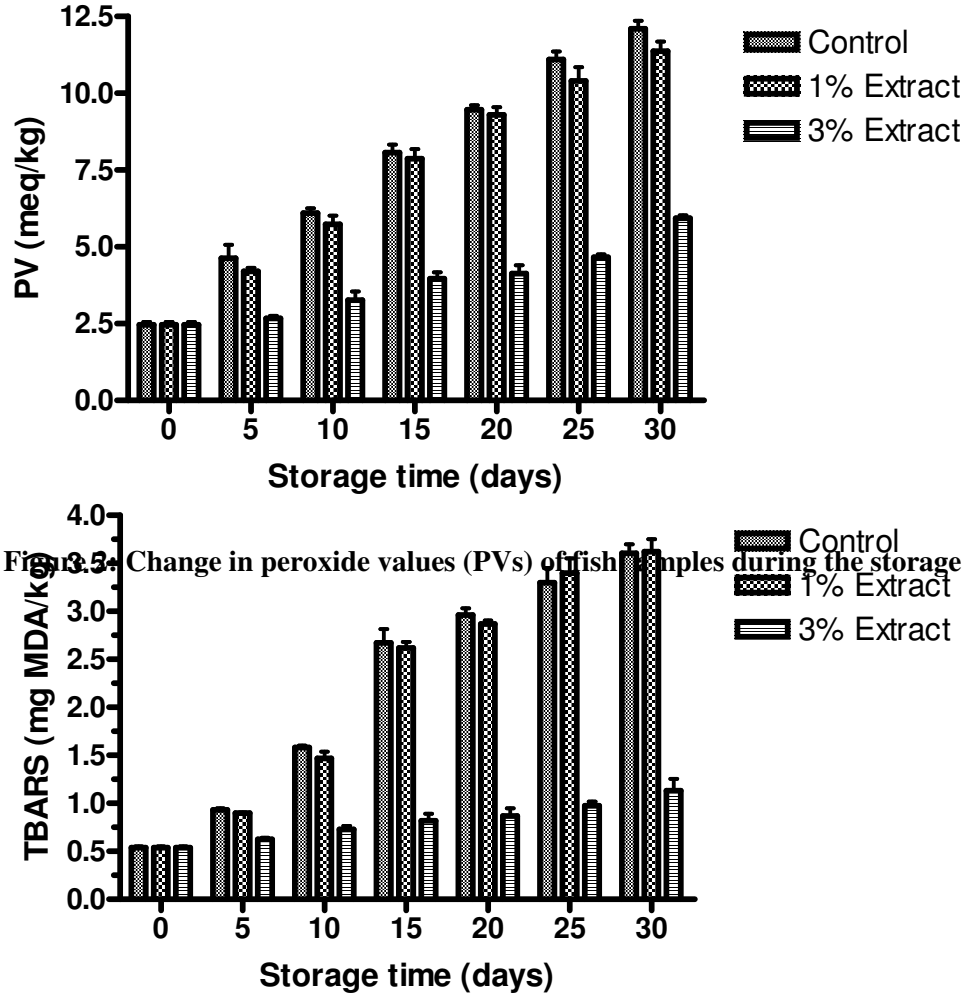


Figure 3: Change in peroxide values (PVs) of fish samples during the storage

Figure 3: Change in TBARS values of fish samples during the storage

Similar to PV results, TBARS values (Fig. 3) in 3% treated sample were lower than the control and 1% treated sample during days 5 until the end of storage period ($p<.001$) and there was no difference between control and 1% treated samples ($p>.05$).

Changes in TVB-N value are shown in Fig. 4. The initial TVB-N value was 9±0.5 mg/100 g and it increased progressively with time of superchilled storage for both treated samples and control.

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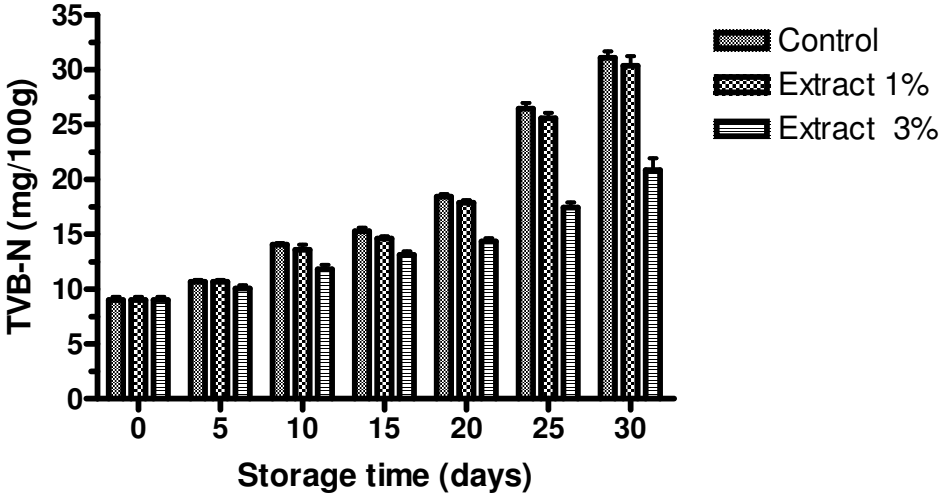


Figure 4: Changes in TVB-N values of fish samples during the storage.

The data showed that TVB-N increase was significantly lower in 3% treated sample than 1% treated one and control from day 10th until the end of the storage ($p<.05$). In this regard, there was no difference between control and 1% treated sample throughout the entire storage period ($p>.05$).

The results of the sensory evaluation of samples are given in Fig. 5. Sensory scores showed a significant decline in both treated and control samples with increasing storage period and, also, the 3% treated sample received a higher score than did the 1% treated sample and control ($p<.05$).

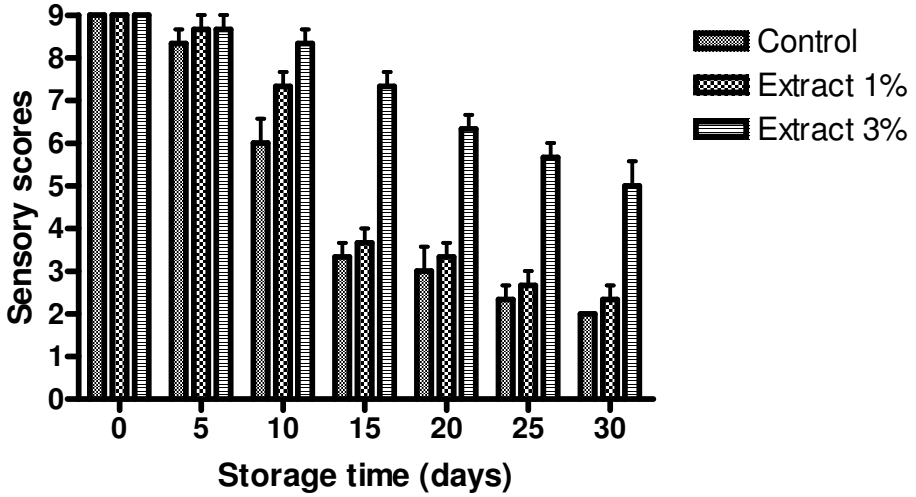


Figure 5: Changes in sensory scores of fish samples during the storage

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Discussion

The initial pH of fillets on day 0 was 6.6 that was higher than those reported by Fan et al (2009) on fresh silver carp fillets (pH=6). In all fish samples, the values of pH decreased initially and then increased. Similar observations were made by Manju et al. (2007). The initial pH decrease was probably due to dissolution of CO_2 in aqueous phase of the fish sample, while the increase of pH was postulated to be due to an increase in volatile bases produced, e.g. ammonia and trimethylamine, by either endogenous or microbial enzymes (Manat et al., 2005). It is concluded that the lower pH of 3% treated sample can enhance microbial inhibition and contributes to the extending of the preservation of fish samples by inhibiting the activity of the endogenous proteases (by *M.pulegium* water extract).

Degradation of polyunsaturated fatty acids (PUFA) by auto-oxidation during storage and the processing of fish oils and fatty fish easily lead to the formation of volatiles associated with rancidity (Taheri et al., 2012). Hydroperoxide is a primary oxidative product of polyunsaturated fatty acids (Erickson., 1997) while The TBARS value is an index of secondary lipid oxidation measuring malondialdehyde (MDA) content. MDA is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez et al., 1997). The concentration of MDA is the direct evidence of toxic processes, caused by free radicals (Duran and Talas., 2009).

At present study, we showed that water extract of *M. pulegium* is able to inhibit both primary and secondary oxidation of dipped fish fillets during storage. While PVs and TBARS levels of the fillets in the control group showed a rapid increase after 5 days of incubation, a slight increase was shown in the 3% treated samples with *M. pulegium* water extract. Peroxide values and TBARS levels of 5 meq/kg and 2 mg/kg of flesh are respectively regarded as the maximal permissible limit in the fish muscle (Ludorff and Meyer., 1973;Connel., 1990). In this study, the initial PV and TBARS values of fresh samples were 2.47 ± 0.15 meq/kg and 0.54 ± 0.02 mg MDA/kg respectively. In control samples these parameters reached to maximal permissible limit after 10 and 15 days in PV (6.1±0.26 meq/kg) and TBARS (2.67±0.2 mg MDA/kg) test respectively; however the final PVs and TBARS values of 3% treated samples were within the limit value after 25 and 30 days respectively from the beginning of the experiment. The data revealed that the *M. pulegium* treated samples indicated preservation of fish flesh by inhibiting the oxidation of lipid.

These characteristics of the water extract of the *M. pulegium* can be attributed to its phenolics, flavonoids and terpenoids constituents. These compounds have been shown in our phytochemical analysis. In this regard, Luximun-Ramma et al. (2002) showed a linear correlation between antioxidant activity and phenolic contents of the plant extracts, fruits and beverages. Sugihara et al. (1999) and Spencer (2008), discussed that flavonoids are able to scavenge hydroxyl radicals,

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superoxide anions and lipid peroxyl radicals, also. Moreover, Joshi et al. (2008) showed a potent antioxidant activity for terpenoids. In this regard, Kamkar et al. (2010) showed that these antioxidative components are more soluble in water in comparison with other polar solvents.

Total volatile basic nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana et al., 1997; Fan et al., 2009).

The initial (day 0) value of 9 mg/100g is higher than the values reported for fresh silver carp (7.3 mg/100g) by Fan et al. (2009). Of course, variation in TVBN values of a particular fish species is related to the fish non-protein nitrogen content, which in turn depends on type of fish feeding, season of catching, fish size as well as other environmental factors. Lastly it is directly related to microbial activity in the fish tissue (Connell., 1990; Debevere and Boskou., 1996; Goulas and Kontominas., 2007). According to Connell (1990), a level of 35–40 mg TVB-N/100 g of fish flesh is usually regarded as spoiled. In this experiment, The final TVB-N values of both treated and control samples did not exceed the upper acceptability limit after 30 days of superchilled storage; however, TVB-N increase in 3% treated sample was significantly lower than control after 30 days of the storage indicating of either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) due to the effect of *M.pulegium*water extract in the fish samples.

The significantly ($p < 0.05$) lower TVB-N values of sample containing *M. pulegium* water extract may be attributed to the antibacterial properties of *M.pulegium* and more specifically to its phenolic constituents (Baydar et al., 2004; Burt., 2004). Given the hydrophobic nature of these phenolic compounds, they are dissolved in the lipid phase of cells interfering with the phospholipid bilayer of the cell membrane, which causes an increase in the permeability and a loss of cellular constituents (Mejlholm and Dalgaard., 2002; Mahmoud et al., 2004). Another possible route for exertion of phenolic compounds antimicrobial activity is the impairment of a variety of enzyme systems and inactivation or destruction of genetic material (Mahmoud et al., 2004).

The sensory qualities of fish samples were evaluated in terms of colour, odour, general acceptability and texture, using a nine-point hedonic scale (1,dislike extremely to 9, like extremely). The fish samples were considered to

be acceptable for human consumption until the sensory score reached 4 (Truelstrup Hansen et al., 1995).

It is well known that fish spoilage gives rise to the subsequent development

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of strongly fishy, rancid and putrid odors, and fish are then clearly rejected for consumption by any sensory panel. Thus, the samples treated by 1% *M.pulegium* water extract and control were acceptable up to 15 days while 3% treated sample was in good and acceptable condition during the entire 30 days of storage. This may be attributed to *M. pulegium´*s functional properties, e.g. antioxidant, antimicrobial and oxygen barrier, and this conclusion is supported by our results of chemical quality analyses.

The results of chemical (pH, PV, TBARS,TVB-N), and sensory evaluation analyses indicate that *M. pulegium* water extract can lead to retention of the good quality characteristics and extension of the shelf life of the dipped silver carp during superchilled storage. In this respect, the sample supplemented with 3% water extract was more potent compared with the 1% one in extending the shelf life of fish fillets.

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