Sensory Analysis of Fish Burgers Containing *Ziziphora clinopodioides* Essential Oil and Nisin: The Effect of Natural Preservatives and Microencapsulation

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ABSTRACT: *The present study aimed to evaluate the effect of free and microencapsulated Ziziphora clinopodioides Essential Oil (ZEO) and Nisin individually and in combination on sensory characteristic and shelf life of fish burger during 20 days of storage at 4±1˚C. Fish burgers were prepared and treated with free and microencapsulated form of ZEO and Nisin in 15 treatments and evaluated by 20 stable semi-trained people using a 9-point hedonic screen method on days 0, 5, 10, 15, 20. The chemical composition of fish burgers was also analyzed at the first day of storage. Results indicated that both microencapsulation and combinational use of ZEO and Nisin improved sensorial scores of treated samples during 20-day storage at 4±1˚C (P< 0.05), and samples containing microencapsulated ZEO and Nisin showed the strongest effect on preserving the sensorial quality of fish burgers.*

KEYWORDS: *Hedonic scale; Sensory analysis; Fish burger; Encapsulation.*

INTRODUCTION

Fish burger is secondary minced fish based food [1, 2]. It is produced from minced fish flesh and is commonly stored and marketed cold [3]. Perishable changes result from microbial growth, protein denaturation [4, 5] and lipid oxidation [3, 6] leading to changes in odor, taste, texture, and color of the product and shelf life decrement during storage period[7].

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Consumption of synthetic additives may be harmful; therefore, consumer's demands are growing for food products with "no chemical additive" or "natural additives." It is predicted that the market for food additives is annually growing about 5.6% (Compound Annual Growth Rate CAGR) until to reach 52.2 Billion (United States dollar) by 2020. In recent years, researchers have focused on the use of natural compounds such as essential oil [10, 11] and lantibiotics [12, 13] as a controller of harmful pathogens and new technologies such as microencapsulation [13, 14] to prolong the shelf life of food products. Citrus aurantium mesocarp extract was used to increase the shelf life of the on the shelf life of rainbow trout[8].

Being natural and accepted by consumers get a lot of natural additives such as ZEO and Nisin, to be used in different products as flavoring or preservative agent [9]. According to *Mirshekari*, 2016 nisin Z and sodium benzoate had Antimicrobial and antioxidant effects in vacuum packed Caspian Kutum (Rutilus frisii) fillet stored at 4◦C[10] Ziziphora clinopodioides, belonging to the family of Lamiaceae, is an endemic species of Iran, which grows in the southeast and central parts of Asia such as, Iran, Afghanistan, and Iraq. Z. clinopodioides is an edible, medicinal plant with short stems (5-16 cm), thin and sharp leaves which are frequently used as an additive in food to convey aroma and flavor [11]. Its major phenolic compounds of ZEO are previously reported as polygon 1,8-cineole, thymol, cymene, carvacrol and limonene [12, 13]. Former studies reported that the ZEO is effective against Gram-negative bacteria (Enterobacter aerogenes, Salmonella Enteritidis) and Gram-positive bacteria (Bacillus cereus, Streptococcus pneumoniae, Staphylococcus aureus)[14]. Healing properties of this plant have been reported by Iranian folk medicine as well. There are many problems with local food production, including the problem of microbiological contamination, which is considered a great concern. A safe and acceptable approach to increase the safety and shelf life of tropical foods is the use of essential oils[2]. they can be the best alternative preservatives for hazardous chemical compounds[15].

Nisin is a small, cationic and hydrophobic AntiMicrobial Peptide (AMPs) and is commercially produced by some strains of *Lactococcus lactic* [16]. This lantibiotic is used individually or in combination with other preservatives in food. It is declared as a GRAS (generally recognized as safe) substances by the FDA (Food and Drug Administration) and is permitted to be used in a variety of food in over 40 countries worldwide [17]. Heat stability and stability in low pH levels are its two important applicable characteristics.

Microencapsulation has become an alternative to reduce the susceptibility to changes caused by external factors such as light, oxygen, and temperature, in addition to being prone to evaporating[18]. Microencapsulation is a useful method to prolong a drug release from dosage forms and to reduce its adverse effect[19]. Encapsulation of preservatives such as Nisin and EOs has been performed by several techniques [20, 21]. Good efficiency and better-controlled release are critical factors for choosing the best method of encapsulation. Former studies suggested different matrixes for entrapment of bioactive compounds including Alginate [22], zein [23], Modified starch [24], and Maltodextrin [25]. Microencapsulation is used to provide protection against the degradative reaction by carrier matrices, prevent loss of volatile compounds and enhance the stability of the core materials [26]. Microencapsulated materials showed better emulsion capacity and stability properties after enzyme treatment as well [24].

To the best of our knowledge, there is a few study about the effect of microencapsulation on sensory properties of different food [27, 28]; nevertheless, there is no study to compare sensory properties of microencapsulated and free preservatives addition of treated fish burgers with ZEO.

Therefore the present study aimed to comparatively evaluate the effect of Nisin and ZEO (microencapsulated and free) individually and in combination with sensory properties of fish burgers during 20 days of storage at 4±1 °C.

EXPERIMENTAL SECTION

Materials

ZEO was extracted by a hydrodistillation method using a Clevenger-type apparatus. Dried aerial parts of the plant were collected from the Mountains of Bojnourd County in North Khorasan province of Iran. The chemical composition of the EO was analyzed by Gas Chromatography-Mass Spectrophotometry (GC-MS) [29]. Nisin was purchased from Sigma-Aldrich Chemical

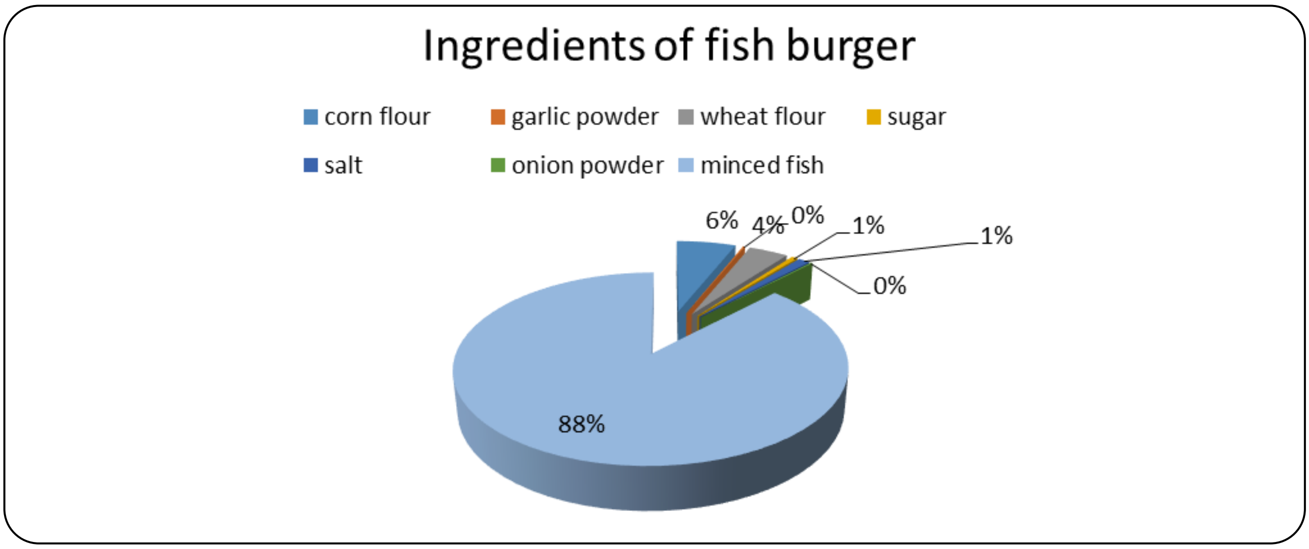

Fig. 1: Ingredients used for the formulation of fish burgers (%).

Co (St. Louis, USA) and prepared by dissolving (50 mg) in 5 ml hydrochloric acid (0.02 Mol/L) to provide standard solutions 200 IU/g.

ZEO and Nisin microencapsulation

ZEO and Nisin were microencapsulated using a mini spray dryer (Buchi B191, Switzerland). Modified starch and Zein were used to catch the ZEO and Nisin microencapsulated powders, respectively according to the method previously described by *Xiao D. et al.* (2011)[30].

Preparations of treatments

Fresh fish were purchased from an aquaculture farm in Bojnourd county, Iran stowed in ice and transferred to the laboratory at the North Khorasan Branch of Academic Center for Education, Culture, and Research (NK-ACECR). All fresh fish were beheaded, gutted and washed. Their flesh was then minced by a meat mincer with a pore size of 1.0 mm. The minced meat was formulated for fish burger preparation according to Fig. 1 [31], mixed well and divided into 15 equal portions as control and treated groups, according to Table 1. Each group was separately packed (each pack: 50 g) in UV-strolled plastics, pressed by hamburger press machine (diameter: 11 cm, thickness: 0.8 cm), frozen at -18 °C for two hours and stored at 4±1˚C for 20 days.

Proximate composition

Fresh fish burgers were analyzed for total protein content by micro-Kjeldahl method [32], total fat content by Soxhlet method [33] and ash and moisture content [34, 35].

Sensory evaluation

Fish burgers were cooked according to the method reported by *Dreeling et al.* (2000) [36]. Briefly, both sides of each fish burger were fried on a hot plate for 10 min until a well done cooked burger was obtained [37]. Samples were fried periodically on days 0, 5, 10, 15 and 20 during storage. Sensory evaluation was carried out using the 9-point hedonic scale on days: 0, 5, 10, 15 and 20 [38, 39]. The panelist (20 semi-skilled members) were firstly trained about the product characteristics; then they scored the samples [40], attributes including color, odor, taste, texture and general acceptability according to the following scale: 1-3: bad or unacceptable, 4-6: good, 7-8: very good, 9: excellent) [38, 41].

Statistical analysis

All sensory indices were described as median (minimum, maximum) for each group. Sensory scores during storage time (every 5 days) were compared among groups, by Kruskal-Wallis Test. Pairwise comparison of experimental groups with the control group and each group with the same pair of the microencapsulated group were performed using Mann-Whitney U test. Since this was multiple testing of the data, the significance level was adjusted at $p<0.01$. All statistical analysis was performed using the SPSS (version 21).

Table 1: List of the treatments of the present study.

Groups/day	ZEO/ Microencapsulated(Mic)*
A	Samples containing no addition (Control)
B	Samples containing Free ZEO 0.25% (w/w)
C	Free ZEO 0.5% (w/w)
D	Free ZEO 1% (w/w)
E	Free Nisin 200 IU mL/kg
F	Samples containing Free ZEO 0.25% (w/w) and Free Nisin 200 IU mL/kg
G	Free ZEO 0.5% (w/w)+ Free Nisin 200 IU mL/kg
H	Free ZEO 1% (w/w)+ Free Nisin 200 IU mL/kg
I	Samples containing Mic ZEO 0.25% (w/w)
J	Mic$^{\beta}$ ZEO 0.5% (w/w)
K	Mic$^{\beta}$ ZEO 1% (w/w)
L	Mic$^{\beta}$ Nisin 200 IU mL/kg
M	Samples containing Mic$^{\beta}$ ZEO 0.25% (w/w)+ Mic Nisin 200 IU/g
N	Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic Nisin 200 IU mL/kg
O	Mic$^{\beta}$ ZEO 1% (w/w)+ Mic Nisin 200 IU mL/kg

$^{\beta}$*Microencapsulated (Mic)*

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of fish burgers was shown in Fig. 2 as follow: protein 16%, fat 7.2%, ash 2%, carbohydrate 7.8%, and moisture 49%, which was similar to the results previously reported by *Ehsani et al.* (2014) [42]. In other studies have also been reported similar results [43, 44]. The higher amount of carbohydrate in fish burgers than fish is due to the presence of flour and sugar in its formulation [45-47].

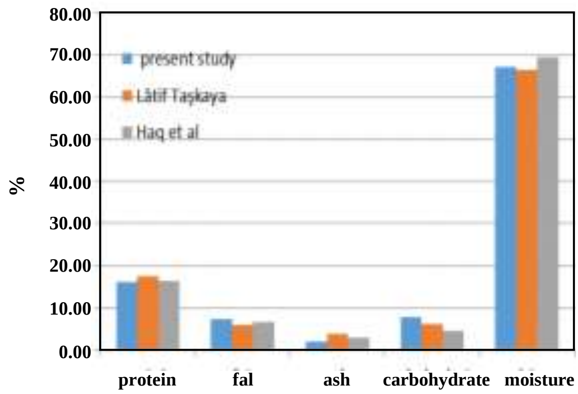

Fig. 2: Proximate analysis of fish burgers among different studies.

Sensory evaluations

According to *Jouki et al.* (2014), high levels of EOs have negative effects on organoleptic properties especially flavor of food [48]; therefore evaluation of sensorial characteristic including taste, texture, odor, color, and overall acceptability are very important in foods containing EO. The level of Food sensory evaluation for each individual is affected by cultural, age, gender, education, economics, social, and urbanization [49]. Combinational use of Nisin and ZEO in the microencapsulated form in fish burgers increased sensory scores of the samples when compared to those with free ZEO and Nisin during storage. Microencapsulation improved sensorial scores of treated samples.

Color attribute

The color is one of the most important characteristics of meat since it is the primary attribute by which both fresh and Obsolescence foods are judged by the consumer before purchase [50]. The color is the main aspect that defines a food's quality, and a product may be rejected simply because of its color, even before other properties, such as aroma, texture, and taste, can be evaluated[51]. Color scores of fish burgers are shown in Table 3 during 20 days of storage at 4±1˚C. The color desirability of

Table 3: Color properties of cooked fish burgers [values are median (min-max).

Groups/day	Zero	5	10	15	20
Control (without Nisin and EO)	9(8-9)	8.6(8-9)	8.2(8-9)	7.5(8-9)	76-9)
Free ZEO 0.25% (w/w)	8.8(8-9)	8.7(8-9)	8.5(8-9)	7.6(7-9)	7(6-9)
Free ZEO 0.5% (w/w)	8.9(8-9)	8.9(8-9)	8.5(8-9)	7.9*(7-9)	7.3*(6-9)
Free ZEO 1% (w/w)	9(8-9)	8.9(8-9)	8.6(8-9)	8* (7-9)	7.5*(6-9)
Free Nisin 200 IU mL/kg	8.8(8-9)	8.7*(8-9)	8.5(8-9)	8* (7-9)	7.4(6-9)
Free ZEO 0.25% (w/w)+ Free Nisin200 IU mL/kg	8.8(8-9)	8.8(8-9)	8.6(8-9)	8.1(7-9)	7.4(6-9)
Free ZEO 0.5% (w/w)+ Free Nisin 200 IU mL/kg	8.9(8-9)	8.8(8-9)	8.6(8-9)	8(7-9)	7.5(6-9)
Free ZEO 1% (w/w)+ Free Nisin 200 IU mL/kg	9(8-9)	8.9(8-9)	8.6(8-9)	8.2(7-9)	7.7(8-9)
Mic$^{\beta}$ ZEO 0.25% (w/w)	8.8(8-9)	8.6(8-9)	8.6(8-9)	8.2(7-9)	7.7(6-9)
Mic$^{\beta}$ ZEO 0.5% (w/w)	8.8(8-9)	8.8(8-9)	8.7(8-9)	8.4*(7-9)	7.9*(6-9)
Mic$^{\beta}$ ZEO 1% (w/w)	8.9(8-9)	8.9(8-9)	8.7(8-9)	8.4*(7-9)	7.8*(6-9)
Mic$^{\beta}$ NISIN 200 IU mL/kg	9(8-9)	9*(8-9)	8.6(8-9)	8.4*(7-9)	7.8(6-9)
Mic$^{\beta}$ ZEO 0.25% (w/w)+ Mic Nisin 200 IU mL/kg	8.9(8-9)	9*(8-9)	8.7(8-9)	8.5*(7-9)	8*(6-9)
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic Nisin 200 IU mL/kg	9(8-9)	9*(8-9)	8.8(8-9)	8.7*(7-9)	8.5*$^{\gamma}$(6-9)
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic NISIN200 IU mL/kg	9(8-9)	9*(8-9)	8.7(8-9)	8.7*(7-9)	8.5*(6-9)

* *Values within a column followed by asterisk (*) are significantly different with control (P < 0.01)*

$^{\gamma}$ *Values for microencapsulated groups followed by Gamma letter (γ)are significantly different with pair non-microencapsulated groups*

$^{\beta}$*Microencapsulated (Mic)*

control samples were scored as 8.8 and 7 at first and last days of storage, respectively. Similar these results were reported by *Tokur et al.* (2006) [52]. Color attribute significantly differed among samples treated with either ZEO or Nisin in free and microencapsulated form; but they were clearly better than control samples during storage, which is completely in agreement with results previously reported by *Ramadhan et al.* (2011) [44].

Odor attribute

In general, the products of freshwater fish are almost odorless [25]. Table 4 shows the results were obtained from odor evaluation of trout fish burgers. As are shown, all treatments had a decrease in acceptability of odor attribute during storage. Samples containing microencapsulated additives received higher scores than control for odor attribute at the end of the storage period ($P< 0.05$), and the highest score was observed in samples mixed with combination use of microencapsulated ZEO and Nisin (M and N groups). Although odor attribute of free ZEO treated groups (ZEO 0.5% and 1%), had higher scores on the first day of storage. The utilization of encapsulated polyphenols, instead of free compounds, can effectively alleviate some deficiencies [53]. Odor refers to the sensation that occurs when volatiles from a product enter the nasal passage and are perceived by the olfactometry system, and these sensations are most often associated with the formulation of compounds during the cooking process[25]. According to these results, the unpleasant taste of the most phenolic compounds is one of the factors, which limit their application at higher concentrations [18, 54]. In this study of odor was limited by microencapsulation method and this groups got better odor score.

Taste attribute

Consumer preferences are linked directly to the human senses sight, touch, smell, taste, and mouthfeel. One major reason, among many, that we like to eat is because food tastes good, which equates to flavor and aroma[25]. Taste score in groups cooperated with free ZEO and Nisin was better than the control group

Table 4: Odor properties of cooked fish burgers [values are median (min-max).

Groups/day	ZERO	5	10	15	20
Con	7.5(7-9)	7.1(7-9)	6.5(6-9)	5.3(5-8)	3.5(3-7)
Free ZEO 0.25%	9* (7-9)	8.7* (7-9)	8* (6-9)	7.3* (5-8)	6.3* (3-7)
Free ZEO 0.5%	8.3* (7-9)	8.1* (7-9)	7.5* (6-9)	7.1* (5-8)	6.1* (3-7)
Free ZEO 1%	7.1(7-9)	7.3(7-9)	6.6(6-9)	6.2(5-8)	6* (3-7)
Free Nisin 200 IU/g	7.5(7-9)	7(7-9)	6.6(6-9)	5.8(5-8)	5.5* (3-7)
Free ZEO 0.25%+ Free Nisin 200 IU/g	9* (7-9)	8.7* (7-9)	8.2* (6-9)	7.5* (5-8)	6.1* (3-7)
Free ZEO 0.5%(w/w)+ Free Nisin 200 IU/g	8.2* (7-9)	8.1(7-9)	7.5* (6-9)	7.3* (5-8)	6.3* (3-7)
Free ZEO 1% (w/w)+ Free Nisin 200 IU/g	7.2(7-9)	7.1(7-9)	6.8(6-9)	6.3(5-8)	6(3-7)
Mic$^{\beta}$ ZEO 0.25%	9* (7-9)	9* (7-9)	8.6* (6-9)	7.8* (5-8)	6.4* (3-7)
Mic$^{\beta}$ ZEO 0.5%	8.7* (7-9)	8.5* (7-9)	8.5*$^{\gamma}$ (6-9)	7.8* (5-8)	6.4* (3-7)
Mic$^{\beta}$ ZEO 1%	8.1(7-9)	7.9(7-9)	7.8* (6-9)	7.1* (5-8)	6* (3-7)
Mic$^{\beta}$ Nisin 200 IU IU/g	7.5(7-9)	7.2(7-9)	6.8(6-9)	6.4(5-8)	5.2* (3-7)
Mic$^{\beta}$ ZEO 0.25%+ Mic Nisin 200 IU/g	9* (7-9)	9*$^{\gamma}$ (7-9)	6.8* (6-9)	7.7(5-8)	6.6* (3-7)
Mic$^{\beta}$ ZEO 0.5%+ Mic Nisin 200 IU/g	8.8* (7-9)	8.6* (7-9)	8.5* (6-9)	7.6* (5-8)	6.5* (3-7)
Mic ZEO 0.5%+ Mic Nisin 200 IU/g	8.1* (7-9)	8(7-9)	7.9*$^{\gamma}$ (6-9)	7.2* (5-8)	6.1* (3-7)

* *Values within a column followed by asterisk (*) are significantly different with control (P < 0.01)*

$^{\gamma}$ *Values for microencapsulated groups followed by Gamma letter (γ)are significantly different with pair non-microencapsulated groups*

$^{\beta}$*Microencapsulated (Mic)*

(*P< 0.05*), and the groups mixed with microencapsulated samples ZEO and Nisin obtained the highest amount of taste score. Due to inappropriate organoleptic characteristics and probable spoilage, Taste evaluation of samples was not performed at 15th and 20th days of storage. As shown in Table 5, a similar pattern of decrease in acceptability of Taste attribute has been observed in all of the investigated sensory attributes during storage [54] but The taste score was shown propriety for consumption by the 10th day, this result Matches with the result of *Leo et al.* (2007) and *Coban et al.* (2016) [19, 41]. However, the reduction rate was lower in all treated samples than the control group, and microencapsulation increased treatments (ZEO and Nisin addition) effect. During the storage period, microorganisms produce organic acids such as lactic acid, which may be it can be considered the reason for this gradual reduction of fish burgers taste scores[55, 56]. The results of the taste evaluation indicated that the most preferred fish burgers were the samples containing the microencapsulated form of either ZEO (0.25 and 0.5%) or Nisin which were significantly different from, other samples up to 10th day of storage.

Texture attribute

A visual evaluation of the texture attributes of a product such as surface smoothness, consistency of texture[25]. Table 5 shows the changes of texture attribute of fish burgers based on panelists evaluation. In this investigation, the Results indicated that through the incorporation of microencapsulated ZEO and Nisin, texture attribute of fish burgers improved and achieved higher scores by panelists. Texture can be defined as the sensory and functional manifestation of the structural and mechanical properties of foods detected through the senses of vision, hearing, touch, and kinesthetic [57]. This result is in line with those reported by *Hassanein et al.* (2013) who reported that addition of soy protein and other non-meat ingredients to the fish burger formulation improved its texture characteristics by an increase of hardness and cohesiveness [58]. In control sample,

Table 5: Taste properties of cooked fish burgers [values are median (min-max).

Groups/day	ZERO	5	10	15	20
Control (without Nisin and EO)	7.5(7-9)	7.1(7-9)	6.1(6-9)	NA	NA
Free ZEO 0.25% (w/w)	8.8* (7-9)	7.6(7-9)	6.5* (6-9)	NA	NA
Free ZEO 0.5% (w/w)	8(7-9)	7.4(7-9)	6.4(6-9)	NA	NA
Free ZEO 1% (w/w)	7(7-9)	6.8(7-9)	6.2(6-9)	NA	NA
Free Nisin 200 IU mL/kg	7.5(7-9)	7(7-9)	6.2(6-9)	NA	NA
Free ZEO 0.25% (w/w)+ Free Nisin200 IU mL/kg	8.7* (7-9)	7.7(7-9)	7.2* (6-9)	NA	NA
Free ZEO 0.5% (w/w)+ Free Nisin 200 IU mL/kg	8(7-9)	7.6(7-9)	7.4* (6-9)	NA	NA
Free ZEO 1% (w/w)+ Free Nisin 200 IU mL/kg	7(7-9)	6.9(7-9)	6.5(6-9)	NA	NA
Mic$^{\beta}$ ZEO 0.25% (w/w)	9* (7-9)	8.6*$^{\gamma}$(7-9)	7.9*$^{\gamma}$ (6-9)	NA	NA
Mic$^{\beta}$ ZEO 0.5% (w/w)	9*$^{\gamma}$ (7-9)	8.7*$^{\gamma}$(7-9)	8*$^{\gamma}$ (6-9)	NA	NA
Mic$^{\beta}$ ZEO 1% (w/w)	8.1* (7-9)	7.8(7-9)	7.5*$^{\gamma}$ (6-9)	NA	NA
Mic$^{\beta}$ NISIN 200 IU mL/kg	7.6(7-9)	7.3* (7-9)	6.4(6-9)	NA	NA
Mic$^{\beta}$ ZEO 0.25% (w/w)+ Mic NISIN200 IU mL/kg	9*$^{\gamma}$ (7-9)	8.7*$^{\gamma}$(7-9)	8.4*$^{\gamma}$ (6-9)	NA	NA
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic Nisin 200 IU mL/kg	9* (7-9)	8.7* (7-9)	8.7* (6-9)	NA	NA
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic Nisin 200 IU mL/kg	8(7-9)	7.7(7-9)	8*$^{\gamma}$ (6-9)	NA	NA

* *Values within a column followed by asterisk (*) are significantly different with control ($P < 0.01$)*

$^{\gamma}$ *Values for microencapsulated groups followed by Gamm letter (γ)are significantly different with pair non-microencapsulated groups*

$^{\beta}$*Microencapsulated (Mic)*

the texture desirability was scored as 8.8 and 3.8, at the first and the last day of storage, respectively.

General acceptability Attribute

The results of the general acceptability attribute of trout Fish Burger are presented in Table 6. The lowest and the highest scores were observed in control sample, and samples with the combinational use of microencapsulated ZEO and Nisin. The results indicated that using ZEO and Nisin especially in microencapsulated form (M, N, O groups) improved the sensory quality of fish burgers. These results are in agreement with *Mahmoudzadeh et al.* (2010) who evaluated sensory scores of fish products treated with rosemary extract and *Carbo et al.* (2009) who investigated about Thymol and modified atmosphere packaging to control microbiological spoilage in packed fresh cod hamburgers [59, 60]. Similarly, *Ojagh et al.* (2010) and *Joki et al.* (2014) reported that the application of biopolymer EOs increased overall acceptability of fish fillets [48, 61]. In sensory evaluation, all quality scores of the samples treated with supplements containing especially in microencapsulated form were in the midrange or higher amounts. The compositional variation may possibly lead to changes in the sensory attributes; including taste, odor, texture, color, and surface appearance, which control the acceptability of fish as food [62] as it may affect the microbial growth [60]. Microencapsulation technique makes a barrier around the oils particles and improves the oxidative stability of the oil. It becomes apparent that combined application of Nisin and ZEO extended the acceptability Attribute of fish burgers during cold storage to 20 days.

CONCLUSIONS

Adding the ZEO and Nisin to fish burger had a positive effect on sensory properties of the samples. In this evaluation test, (nisin+ZEO) microencapsulated-treated samples showed high acceptability in all of the studied sensory characteristics including odor, color, texture and overall acceptability. By microencapsulation technique, uses of high concentrations of natural

Table 5: Texture properties of cooked fish burgers [values are median (min-max).

Groups/day	ZERO	5	10	15	20
Control (without Nisin and EO)	8.8(8.5-9)	7.5(7.5-9)	6.3(6-9)	5.2(5-8)	3.8(3.5-7)
Free ZEO 0.25% (w/w)	8.8(8.5-9)	8.2(7.5-9)	7.6* (6-9)	6.1* (5-8)	3.8(3.5-7)
Free ZEO 0.5% (w/w)	8.6(8.5-9)	8.3* (7.5-9)	7.6* (6-9)	6.2* (5-8)	4.2(3.5-7)
Free ZEO 1% (w/w)	8.8(8.5-9)	8.5* (7.5-9)	7.2(6-9)	6.7* (5-8)	4.4(3.5-7)
Free Nisin 200 IU mL/kg	8.8(8.5-9)	8.3* (7.5-9)	7.5* (6-9)	6.9* (5-8)	5.1(3.5-7)
Free ZEO 0.25% (w/w)+ Free Nisin 200 IU mL/kg	8.7(8.5-9)	8.4* (7.5-9)	7.8* (6-9)	6.3* (5-8)	4.5(3.5-7)
Free ZEO 0.5% (w/w)+ Free Nisin 200 IU mL/kg	8.9(8.5-9)	8.7* (7.5-9)	7.3* (6-9)	6.2* (5-8)	5.3* (3.5-7)
Free ZEO 1% (w/w)+ Free Nisin 200 IU mL/kg	8.6(8.5-9)	8.5* (7.5-9)	7.5* (6-9)	6.5* (5-8)	5.7* (3.5-7)
Mic$^{\beta}$ ZEO 0.25% (w/w)	8.8(8.5-9)	8.6* (7.5-9)	7.6* (6-9)	7.5$^{*\gamma}$ (5-8)	4.7* (3.5-7)
Mic$^{\beta}$ ZEO 0.5% (w/w)	8.8(8.5-9)	8.7* (7.5-9)	8.4$^{*\gamma}$ (6-9)	7.6$^{*\gamma}$ (5-8)	5.7$^{*\gamma}$ (3.5-7)
Mic$^{\beta}$ ZEO 1% (w/w)	8.9* (8.5-9)	8.9* (7.5-9)	8.5$^{*\gamma}$ (6-9)	7.6* (5-8)	5.8$^{*\gamma}$ (3.5-7)
Mic$^{\beta}$ Nisin 200 IU mL/kg	9* (8.5-9)	8.9* (7.5-9)	8.5$^{*\gamma}$ (6-9)	7.5* (5-8)	5.8*(3.5-7)
Mic$^{\beta}$ ZEO 0.25% (w/w)+ Mic NISIN200 IU mL/kg	9(8.5-9)	8.9* (7.5-9)	8* (6-9)	7.3$^{*\gamma}$ (5-8)	6.2$^{*\gamma}$ (3.5-7)
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic Nisin 200 IU mL/kg	9(8.5-9)	8.9* 7.5-9)	8.2* (6-9)	7.7$^{*\gamma}$ (5-8)	6.4* (3.5-7)
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic Nisin 200 IU mL/kg	8.9(8.5-9)	8.9* (7.5-9)	8.2$^{*\gamma}$ (6-9)	7.8$^{*\gamma}$ (5-8)	6.2* (3.5-7)

* *Values within a column followed by asterisk (*) are significantly different with control ($P < 0.01$)*

$^{\gamma}$ *Values for microencapsulated groups followed by Gamma letter (γ) are significantly different with pair non-microencapsulated groups*

$^{\beta}$*Microencapsulated (Mic)*

Table 6: Acceptability properties of cooked fish burgers [values are median (min-max).

Groups/day	ZERO	5	10	15	20
Control (without NISIN and EO)	7.95(7.5-9)	7.57(7.5-9)	6.77* (6.5-9)	6(6-8.5)	4.76(4.5-7.5)
Free ZEO 0.25% (w/w)	8.8(7.5-9)	8.3(7.5-9)	7.65(6.5-9)	7(6-8.5)	5.63(4.5-7.5)
Free ZEO 0.5% (w/w)	8.37(7.5-9)	8.17 (7.5-9)	7.67* (6.5-9)	7.06(6-8.5)	5.93*(4.5-7.5)
Free ZEO 1% (w/w)	7.97(7.5-9)	7.85(7.5-9)	7.96* (6.5-9)	6.96(6-8.5)	5.96*(4.5-7.5)
Free NISIN 200 IU mL/kg	8.15(7.5-9)	7.82(7.5-9)	7.32(6.5-9)	5.9(6-8.5)	5.9(4.5-7.5)
Free ZEO 0.25% (w/w)+ Free NISIN200 IU mL/kg	8.8(7.5-9)	8.6* (7.5-9)	7.9* (6.5-9)	7.3* (6-8.5)	6* (4.5-7.5)
Free ZEO 0.5% (w/w)+ Free NISIN 200 IU mL/kg	8.45(7.5-9)	8.3* (7.5-9)	7.7(6.5-9)	6.9(6-8.5)	6.36*(4.5-7.5)
Free ZEO 1% (w/w)+ Free NISIN200 IU mL/kg	7.97(7.5-9)	7.92(7.5-9)	7.4(6.5-9)	7.1(6-8.5)	6.46*(4.5-7.5)
Mic$^{\beta}$ ZEO 0.25% (w/w)	8.9* (7.5-9)	8.7* (7.5-9)	8* (6.5-9)	7.8* (6-8.5)	6.13*(4.5-7.5)
Mic$^{\beta}$ ZEO 0.5% (w/w)	8.82(7.5-9)	8.67* (7.5-9)	8.4$^{*\gamma}$ (6.5-9)	7.93$^{*\gamma}$ (6-8.5)	6.6$^{*\gamma}$(4.5-7.5)
Mic$^{\beta}$ ZEO 1% (w/w)	8.5(7.5-9)	8.37(7.5-9)	8.05* (6.5-9)	7.7* (6-8.5)	6.66*(4.5-7.5)
Mic$^{\beta}$ NISIN 200 IU mL/kg	8.27(7.5-9)	8.1(7.5-9)	7.55(6.5-9)	7.36$^{*\gamma}$ (6-8.5)	6.33* (4.5-7.5)
Mic$^{\beta}$ ZEO 0.25% (w/w)+ Mic NISIN200 IU mL/kg	8.95$^{*\gamma}$ (7.5-9)	8.47(7.5-9)	8.83$^{*\gamma}$ (6.5-9)	7.83* (6-8.5)	7.13$^{*\gamma}$ (4.5-7.5)
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic NISIN200 IU mL/kg	9$^{*\gamma}$ (7.5-9)	8.95* (7.5-9)	8.6$^{*\gamma}$ (6.5-9)	8* (6-8.5)	7.36$^{*\gamma}$ (4.5-7.5)
Mic$^{\beta}$ ZEO 0.5% (w/w)+ Mic NISIN200 IU mL/kg	8.47	8.45(7.5-9)	8.25* (6.5-9)	8.1* (6-8.5)	7.5* (4.5-7.5)

* *Values within a column followed by asterisk (*) are significantly different with control ($P < 0.01$)*

$^{\gamma}$ *Values for microencapsulated groups followed by Gamma letter (γ)are significantly different with pair non-microencapsulated groups*

$^{\beta}$*Microencapsulated (Mic)*

preservatives such as Eos is feasibleness for their desired antimicrobial effects and can be used for increasing the shelf life of food during the storage period; Because they may convey unpleasant sensory effects especially on the flavor and taste of foods in free form. In the present study, an addition of a microencapsulated form of ZEO and Nisin could increase sensory scores of fish burgers when compared to control and treatments containing no-microencapsulated form of ZEO and Nisin.

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