JFBE 5(2): 99-108, 2022

Journal of Food and Bioprocess Engineering

Journal homepage: https://jfabe.ut.ac.ir

Original research

Development of fibrous casings with natural antioxidants using rosemary and thyme extracts in dried sausages

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A B S T R A C T

In this research, an active packaging system with antioxidant properties has been designed for the shelf life extension of sausage. Cotton linter-based fibrous casing has been coated with thyme and rosemary extract with different percentages (0, 2, 4, and 6%). The water vapor permeability rate, extract loading on the casing, total phenolic content, DPPH radical scavenging activity and migration of phenolic compounds were determined in developed casings. Lipid oxidation (TBARS), film color and sensory properties of packed sausage were assessed during 2 months in cold storage (4 ± 1°C). Casings containing 0 and 2% of thyme and rosemary extract showed the most hydrophobic and the lowest water vapor permeability. Phenolic compounds migration was different from 60 to 191 (mg Gallic acid /g) and the result showed that releasing extent and rate of both extracts were significant ($p < 0.05$). Fibrous casing loaded with 6% thyme extract showed the highest antioxidant activity (1.01 mg malondialdehyde/ kg). Sausage packaged in a fibrous casing loaded with 6% thyme extract was preferred by the panelists in terms of overall acceptability. Regarding Sausage color, there was no significant difference between active and control packaged sausage samples ($p > 0.05$). There was a significant difference in color between extract loaded and control casings ($p < 0.05$). It was concluded that loading fibrous casing with natural antioxidant extract could be introduced an active antioxidant packaging which effectively can reduce fat oxidation in meat products.

Keywords: Herbal extracts; Active packaging; Fibrous casing; Sausage

Received 11 June 2022; Revised 03 July 2022; Accepted 05 July 2022

1. Introduction

Active packaging is one of the new concepts in food packaging that has been developed in response to the constant changes in current consumer demands and market trends. The main techniques used in active packaging include adsorbing/scavenging (oxygen, ethylene, moisture, carbon dioxide, and odors/flavors), emitting/releasing (carbon dioxide, antimicrobials, antioxidants), and incorporation of some active agent in package headspace or packaging materials (Firouz et al., 2021). Several studies have demonstrated the effectiveness in the use of plant extracts and essential oils as part of the packaging material. Additionally, it is well known that some natural extracts presented both, antimicrobial and antioxidant activities, which allows double protective function. To this regard, multiple researches found both activities in plant extracts (Kiarsi et al., 2020). One of the most important types of active packaging is antioxidant packaging which prevents oxidative spoilage by releasing antioxidants during the storage time of fatty foods (Brody et al., 2008). The natural extracts of many plants have been used to increase the shelf life of foods. Plant extracts are kinds of substances that have been integrated into polymers for the preparation of active packages (Gómez-Estaca et al., 2014). Furthermore, the application of natural ingredients is a new trend in the food industry and food research. It means that natural preservatives can be derived from natural sources, instead of synthetic substances and the reduction of additives in foods. Rosemary and thyme extract due to the high content of phenolic compounds by donating electrons to the oxidative chain break this chain and reduce oxidation and increase the shelf life of food (Barbosa-Pereira et al., 2014; Sharififar et al., 2007). Studies have shown that many plants in the mint family, such as thyme, and

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https://doi.org/10.22059/jfabe.2022.344339.1117

rosemary, have high phenolic compounds and antioxidant effects (Darie-Niţă et al., 2018). Sharififar et al. (2007) reported the main ingredients of thyme extract is thymol (37.59%), carvacrol (33.65%), paracetamol (72.7%) and gamaterpinen (3.88%). Rosemary extract, due to its compounds such as resmanone, quinone, carnosol and carnosic acid, breaks the free radical scavenging chain by giving hydrogen atoms and delays oxidation (Fernandez-Lopez, 2005). Dried sausages have low oxidative stability and are sensitive to fat sharpening during storage. The phenomenon of oxidation in sausages causes the loss of biologically active compounds. It also results in the accumulation of toxic and carcinogenic substances such as hydroperoxides, radicals, and epoxides (Halagarda & Wójciak, 2022).

Vermin et al. (1999) reported that polymers coated with antioxidant compounds enter the food slowly and reduce oxidation during storage. Whey protein concentrate films incorporated with a blend of *Cinnamomum cassia*, *Cinnamomum zeylanicum*, and *Rosmarinus* essential oils were characterized and their effectiveness as an antioxidant for food application has been investigated (Ribeiro-Santos et al., 2018). The Shelf life of fresh beef steaks wrapped in an antioxidant film with a natural antioxidant coating was examined by Camo *et al.* (2011) which revealed that the presence of oregano extract in the antioxidant packaging showed remarkable oxidative stability. Tátraaljai *et al.* (2014) investigated the ability of polyethylene film containing barley bran extract to prevent spoilage. They concluded that a film containing barley bran extract prevented oxidation and hydrolysis of frozen fish fat. Coating chitosan film with cinnamon oil in salmon improved its chemical, microbial and sensory properties during cold storage and its quality and durability (Ojagh et al., 2010). Another investigation showed that chitosan film coating combined with bamboo vinegar prevents lipid oxidation, thiobarbituric acid production, and it also protects color in prepared to cook meat products (Zhang et al., 2018).

The objective of this research was to develop an active fibrous casing for dried sausage with antioxidant properties which is coated with *Zataria multiflora* (Thyme) and rosemary extract and evaluate the lipid oxidative stability and sensory properties of packaged sausage during storage.

2. Material and Methods

2.1. Materials

Thyme and rosemary extracts were bought from the local market in Tehran suburb and were dried at room temperature for one week. Linter cotton (α- Cellulose) was purchased from Nano Novin Polymer Co. (Gorgan, Iran). Sodium hydroxide, urea, sulfuric acid was prepared from Neutron Co. (Tehran, Iran). Carbon disulfide was obtained from Merck (Germany). Folin-Ciocalteu, Gallic acid, 2-thiobarbituric acid (TBA), and trichloroacetic acid were purchased from Sigma Aldrich.

2.2. Preparation of extract

Firstly, the impurities (wood, stems, wastes) were separated from the leaves (rosemary and thyme) and dried at room temperature and powdered by a blender and sifted with a 40 mesh sieve. The dried and rosemary and thyme leaves powder were put in a cellulose thimble clogged with cotton and inserted into the Soxhlet apparatus, which was fitted with a reflux condenser and placed on a distillation flask containing ethanolic solution [ethanol/water (70:30, v/v)] and boiling glass regulator. Then, the extraction was operated by using a digital four-cell Soxhlet device (BUCHI, Switzerland) at 70°C for two hours (20 g sample, in 200 ml of 70% ethanol). The extracts were concentrated by rotary evaporation aid (HEIDOLPH, Germany) at 40°C. The dried extracts were poured into a dark glass jar and stored in a refrigerator at 4°C until the using time (Tavassoli et al., 2011).

Table 1. The migration of the phenolic compounds from rosemary and thyme extract loaded casing into food simulant.

Type of extract	Total phenol compounds of the extract (mg Gallic acid/g)	IC_{50} (µg/ml)	The amount of dried extract on the film(mg/dm^2)	Extract concentration (%)	Time (h)	Migration of phenolic compounds (mg Gallic acid /g)
Thyme extract	224.80 ± 0.5	677.90 ± 0.9	5.50 ± 0.4^a	2	24	95±0.05^a
				2	48	110±0.03^b
				2	72	110±0.01^b
			7.30 ± 0.5^b	4	24	150±0.02^a
				4	48	170±0.04^b
				4	72	171±0.07^b
			9.10 ± 0. 1^c	6	24	185±0.01^a
				6	48	191±0.04^b
				6	72	191±0.03^b
Rosemary extract	185.30 ± 0.55	986.0 ± 0.9	3.50 ± 0.3^a	2	24	60±0.04^a
				2	48	80±0.03^b
				2	72	81.1±0.03^b
			4.80 ± 0. 2^b	4	24	95±0.05^a
				4	48	110±0.02^b
				4	72	111.0±0.02^b
			6.50 ± 0.2^c	6	24	120±0.03^a
				6	48	140.5±0.04^b
				6	72	141.7±0.05^b

Values quoted are mean values ± standard deviations of results for three experiments. Means with the same letter in each column are not significantly different ($p < 0.05$).

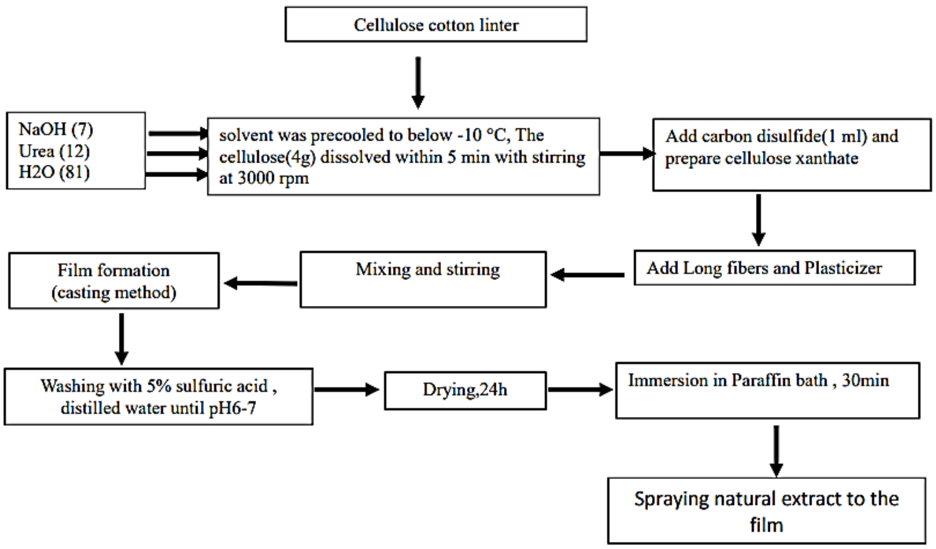

Fig.1. The Overall process proposed for the production of fibrous casings coated with natural antioxidant of rosemary and thyme extract.

Table 2. TBARS (mg malondialdehyde/kg) in sausages packaged with fibrous casing coated with different concentrations of thyme and rosemary extracts.

Casing	Storage time (day)				
Thyme extract (%)	0	15	30	45	60
0	3.34±0.02^{Ea}	3.08±0.02^{Da}	1.90±0.01^{Ca}	0.56±0.01^{Ba}	0.001±0.01^{Aa}
2	2.22±0.01^{Eb}	1.86±0.01^{Db}	1.73±0.03^{Cb}	0.42±0.01^{Bb}	0.001±0.01^{Aa}
4	2.0±0.02^{Ec}	1.69±0.01^{Dc}	1.55±0.01^{Cc}	0.39±0.02^{Bc}	0.001±0.01^{Aa}
6	1.01±0.02^{Ed}	0.95±0.01^{Dd}	0.89±0.02^{Cd}	0.2±0.01^{Bd}	0.001±0.01^{Aa}
Rosemary extract (%)					
0	3.34±0.02^{Ea}	3.08±0.02^{Da}	1.90±0.01^{Ca}	0.56±0.01^{Ba}	0.001±0.01^{Aa}
2	2.55±0.01D^{Eb}	1.90±0.01^{Db}	1.85±0.01^{Cb}	0.48±0.01^{Bb}	0.001±0.01^{Aa}
4	2.04±0.02^{Ec}	1.79±0.01^{Dc}	1.74±0.02^{Cc}	0.40±0.02^{Bc}	0.001±0.01^{Aa}
6	1.22±0.02^{Ed}	1.01±0.02^{Dd}	0.99±0.02^{Cd}	0.34±0.01^{Bd}	0.001±0.01^{Aa}

Values quoted are mean values ± standard deviations of results for three experiments. For each casing in the same column, different superscript letters (a, b, c) means that values are significantly different ($p < 0.05$). For each casing in the same row, different superscript letters (A, B, C) means that values are significantly different ($p < 0.05$).

Table 3. Color parameters of casings loaded with different concentration of thyme and rosmary extract.

Casing	L*	a*	b*	ΔE
Thyme extract (%)				
0	0	6.1±0.40^{a}	-1.80±0.10^{a}	92.34±0.60^{a}
2	0.16±50^{a}	6.0±0.30^{b}	-1.72±0.17^{b}	92.44±0.70^{b}
4	0.51±0.51^{b}	5.6±0.40^{c}	1.79±0.08^{c}-	92.98±0.62^{c}
6	1.27±0.26^{c}	5.5±0. 20^{d}	-1.82±0.09^{d}	93.40±0.68^{d}
Rosemary extract (%)				
0	0	6.1±0.44^{a}	-1.8±0.10^{a}	92.34±0.6^{a}
2	0.20±0.53^{a}	5.98±0.40^{b}	-1.73±0.90^{b}	92.49±0.59^{b}
4	0.45±0.62^{b}	5.83±0.37^{c}	-1.62±0.01^{c}	92.66±0.72^{c}
6	0.64±0.20^{c}	5.72±0.21^{d}	-1.59±0.08^{d}	92.82±0.55^{d}

Results are shown reported as mean ± standard deviation. For each casing in the same column, different superscript letters (a, b, c) means that values are significantly different ($p < 0.05$). For each casing in the same row, different superscript letters (A, B, C) means that values are significantly different ($p < 0.05$).

Table 4. The color changes (ΔE) of the sausage packages with rosemary and thyme extract loaded casings during storage time (4 ± 1°C).

Casing	Storage time (day)				
Thyme extract (%)	0	15	30	45	60
0	0	0	0	0	0
2	56.27±0.88^{Aa}	53.27±0.88^{Aa}	53.27±0.98^{Aa}	50.23±0.98^{Aa}	50.23±0.98^{Aa}
4	56.21±0.60^{Aa}	53.63±0.60^{Aa}	53.62±0.40^{Aa}	50.63±0.60^{Aa}	50.63±0.60^{Aa}
6	57.34±0.43^{Aa}	53.63±0.43^{Aa}	53.63±0.68^{Aa}	50.63±0.68^{Aa}	50.63±0.68^{Aa}
Rosemary extract (%)					
0	0	0	0	0	0
2	52.34±0.91^{Aa}	51.63±0.91^{Aa}	51.63±0.76^{Aa}	50.63±0.76^{Aa}	50.63±0.76^{Aa}
4	55.23±0.60^{Aa}	53.87±0.60^{Aa}	50.87±0.82^{Aa}	50.87±0.60^{Aa}	50.87±0.60^{Aa}
6	57.33±0.56^{Aa}	54.63±0.56^{Aa}	54.63±0.71^{Aa}	50.63±0.45^{Aa}	50.63±0.45^{Aa}

Results are shown reported as mean ± standard deviation. For each casing in the same column, different superscript letters (a, b, c) means that values are significantly different ($p < 0.05$). For each casing in the same row, different superscript letters (A, B, C) means that values are significantly different ($p < 0.05$).

2.3. Film preparation

Films were elaborated following the methodology explained in U.S. Pat. No. 005747125A and Cai and Zhang (2006). Briefly, the aqueous solution containing NaOH/ urea and H_2O at the ratio of 7:12:81 (wt%) were used as a solvent for cellulose regeneration. The solvent was precooled to below -10°C. At this stage, sodium cellulose (Cellulose cotton linter) aerogel was prepared. Cellulose xanthate is then prepared by adding carbon disulfide. To reinforce cellulose xanthate, long cotton fibers and plasticizer (Glycerol) were added, and the film was prepared by casting method. The three stages of the fibrous casing preparing were washing by acid and water, drying, and immersion in plasticizer, respectively. Rosemary and thyme extracts in different concentrations (0, 2, 4, and 6%) were prepared using 70% ethanol as solvent. The concentrations were selected according to the antioxidant activity of the extract concentration (rosemary and thyme) in comparison with the synthetic antioxidant BHA. The antioxidant effect of 4% concentration of thyme and rosemary extracts with 0.02% concentration of synthetic antioxidant BHA was not significantly different. The IC_{50} values in thyme, rosemary and BHA were 19.55, 20.22 and 18.64, respectively. In the next step, fibrous casings were cut in 14 × 14 cm^2 dimensions and the extracts (rosemary and thyme) were sprayed on the films with different concentrations (0, 2, 4 and 6%) (López-de-Dicastillo et al., 2012). After films drying (in a dark place) the sausages were wrapped in fibrous casings (Fig. 1).

2.4. Sausage preparation

The sausage (60%) was purchased from Pak Teliseh food Industries (Tehran, Iran). Sausage formulations contained of meat (60%), soy isolate (2%), ice (22%), spices (0.95%), liquid oil (5%), onion (3%), starch (5%), nitrite Sodium (0.05%), ascorbic acid (0.04%), polyphosphate (0.03%) and salt (1.7%). The cooking operation was performed in the cooking room using water vapor for 70 min until the internal temperature of the product reached 75°C. After cooking, the temperature of the product was cooled down by a cold shower until it reached a temperature of 20°C. Samples were stored in cold storage at 4°C for various experiments.

2.5. Water vapor transmission rate (WVTR)

In summary, the film was tested on a cup containing 12 ml of distilled water. Within 2 hours, steady state conditions were assumed to have occurred. The cells were stored in a temperature and humidity controlled room (a/c unit supplied by Denco Ltd., Herts. UK; conditions: 50 ± 5% relative humidity, 23 ± 2°C), and a fan set at an air velocity of 154 mm/min which was placed over the cells to ensure uniform movement of air. Eight weight measurements were then recorded over a 24 h period with intervals of greater than 1.5 h between readings. At least three replicates of the film samples were tested. Water vapor transmission rate was measured, and permeance and WVTR were calculated, by using the ASTM-E 96 method.

2.6. Extract loading on the films

Extracts loading on the films determined by the difference in weight of the films before and after coating with the extracts was measured. This value as remained extract on the films was expressed in mg/dm^2 (Contini, 2012).

2.7. Quantification of total phenolic compounds

Total phenolic content was determined by the Folin-Ciocalteu method (Mensor et al., 2001). Gallic acid was used as the standard, and samples were read in triplicate at 765 nm in a spectrophotometer (CECIL, Czech Republic). 0.5 ml of the extracted solution was mixed with 2.5 ml of the Folin-Ciocalteu reagent (10-fold diluted). After 3 min of mixing, 2 ml of the 7.5% sodium carbonate solution was added. The absorbance (Spectrophotometer (UV-VIS) CECIL Made in the Czech Republic) was read at 765 nm using a blank of ethanol. The total phenolic content was expressed in mg equivalents of Gallic acid (GAE)/g of each fraction.

2.8. *DPPH radical scavenging activity*

Determination of the free radical scavenging of the thyme and rosemary extracts was performed by the DPPH method (Mensor et al., 2001). Briefly, the DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution (200 µM) was mixed with various concentrations of samples. The absorbance was observed at 517 nm after 30 min of remaining in darkness at room temperature. The DPPH quenching ability was expressed as IC_{50} (the extract concentration required to inhibit 50% of the DPPH in the assay medium). IC_{50} values were obtained based on the linear regression equation for rosemary and thyme extracts.

2.9. *Migration analysis*

The phenolic compounds migration from the films to the food simulant (ethanol 95%) was measured by colorimetric method using Folin-Ciocalteau (Corrales et al., 2009). First, 1 dm^2 of fibrous films containing different concentrations of the extract as well as the control film (film without extract) separately in closed tubes containing 3 ml of 95% ethanol so that the ethanol is in contact with the film. Then all the tubes with caps were placed in the dark at a temperature of 4°C. Migration rates were measured at 24, 48 and 72 h. Using the standard curve, the total amount of phenolic compounds migrated from 1 dm^2 of film to ethanol was obtained in terms of (mg Gallic acid /g) (Tafreshi et al., 2013).

2.10. *Measurement of fat oxidation*

Thiobarbituric acid reactive substances (TBARS) assay was based on the spectrophotometric measurement of a complex formed by the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), according to the method which has been described by Fasseas et al. (2008). In brief, 2 g of each sausage sample was mixed with 1 ml H_2O and 1.5 ml of 20% trichloroacetic acid. It was then filtered with filter paper (Whatman No. 41). 5 ml of sausage trichloroacetate extract was mixed with 5 ml of 0.01 M thiobarbituric acid solution and placed in a 100°C water bath for 1 h to give a color. The absorbance was determined at 532 nm. The TBARS was calculated from the following Eq. (1):

$$\mathrm{TBARS} = \frac{(\mathrm{As} - \mathrm{Ab}) \times 50}{\mathrm{m\ (g)}} \quad (1)$$

As=Sample absorption rate, Ab= Blank absorption rate, m= Sample weight.

2.11. *Film color*

Film color analysis was carried out using a colorimeter (model TES-135, Taiwan). The data were obtained using easy RGB software and were converted to CIE system as L*, a* and b* parameters. The color values were expressed as L*, as lightness, and b*, as yellowness and a*, as redness. Total color difference (ΔE) between the films containing the extract and the film without the extract was calculated by following Eq. 2 (Pedreschi et al., 2007):

$$\Delta E = ((L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2)^{\frac{1}{2}} \quad (2)$$

2.12. *Sensory evaluation*

Sensory evaluation of sausages was performed by using hedonic test (Quesada et al., 2016). Ten trained judges evaluated the samples for odor, color, flavor, texture and overall acceptance. Samples of 8 treatments were placed in 4 cm thick slices in disposable plates (without color and odor). Evaluation was performed at room temperature and under fluorescent light irradiation. Tap water was available for use between testing samples. The five point hedonic scale was designed from 1-5 and range of scores with the level of acceptability were not acceptable, slightly acceptable, moderately acceptable and acceptable and highly acceptable, respectively.

2.13. *Morphological properties*

The surface morphology of the fibrous casings with natural antioxidant films was studied using a scanning electron microscope (SEM, Cambridge Scientific Instrument, S360, England) (Priyadarshi et al., 2021). The SEM was operated at an acceleration voltage of 15 kV. The films were sputter-coated with platinum using a Hitachi MC1000 vacuum sputter coater.

2.14. *Statistical analysis*

Results were expressed as the mean and standard deviation of three independent replicates. All the data were statistically analyzed using one-way analysis of variance (ANOVA) through Duncan post hoc at $p < 0.05$. All of the statistical analyses were performed using Minitab software version 16.

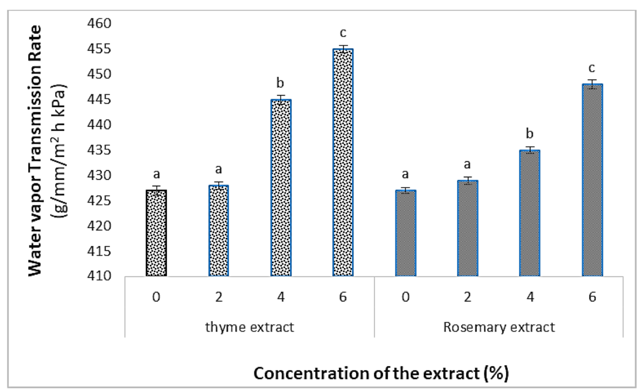

Fig. 2. The effect of rosemary and thyme extract loading content on the water vapor transmission rate of fibrous casings. (Results are reported as mean ± standard deviation. For each extract, the averages with different letters are significantly different).

3. Results and Discussion

3.1. *WVTR*

Fig. 2 displays the influence of the different levels of rosemary and thyme extracts on the WVTR of fibrous films. The compared data of the average data in terms of permeability of fibrous films coated with different percentages of rosemary and thyme extracts are presented in Fig. 2. As shown in Fig. 2, it is clear that with increasing the percentage of rosemary and thyme extract in the fibrous film formulation, the permeability of the films to water vapor increases significantly ($p < 0.05$). However, there is no significant difference between the water vapor permeability of fibrous films containing 2% extract, statistically ($p > 0.05$). The water vapor permeability of films depends on the presence of hydrophilic and hydrophobic compounds and their interaction in the film matrix (McHugh, 1994). Probably due to the presence of significant amounts of hydrophilic compounds such as phenolic compounds in rosemary and thyme extracts, the vapor permeability of fibrous films has been increased. The rupture of the structure and the reduction of the compaction of the film structure due to the addition of the extract can be a reason for increasing the water vapor permeability of the active film (Hosseini et al., 2009). Also, due to the hydrophilic nature of the extract, increasing the hydrophilic substance in the film has a direct impact on the permeability to water vapor. On the other hand, Ribeiro-Santos et al. (2018) stated that water plays the role of plasticizer, and can reduce the intermolecular bonds of polymer chains, so that, water penetration through polymer chains increases. Pruneda et al. (2008) reported that the use of oregano extract in the preparation of edible films based on soy protein isolate increases the water vapor permeability of the resulting films. The researchers said that the increment in water vapor permeability of edible films is due to the presence of hydrophilic compounds such as polyphenolic and flavonoid compounds. Sandhu et al. (2019) has investigated the water vapor permeability of chitosan-based films containing thyme essential oil and found that the water vapor transfer rate was increased compared to the control sample.

3.2. *Migration analysis*

Table 1 shows the phenolic compounds migration values into the food simulant. Over time, the migration rate of phenolic compound has been increased by the augmentation of the rosemary and thyme concentration ($p < 0.05$). With the raising of the extracts concentration from 2 to 6%, the rate of migration to ethanol increased significantly ($p < 0.05$). The migration rate of thyme extract phenolic compounds at all tested levels was higher than the rosemary extract. According to the study of Lin et al. (2009) in the thyme extract due to the presence of polar compounds such as thymol and carvacrol more migration happened into the food simulant environment.

3.3. *Measurement of fat oxidation*

The oxidation of packaged sausages in the fibrous casing loaded with different concentrations of thyme and rosemary extracts are presented in Table 2. Thyme and rosemary extracts loading in casing caused significant differences in fat oxidation results ($p < 0.05$). The hydrophilic nature of extract could have an influence on the properties of polymer based films enriched with extract due to increase the water sorption capacity of the films (Ojagh et al., 2010). Fat oxidation increased in all samples during storage period. The highest rate of fat oxidation was related to the control sample (casing without extract) and the lowest was related to the fibrous coating loaded with 6 % thyme extract. The higher concentration of rosemary and thyme extracts loading on the casing films is resulted the lower fat oxidation in packaged sausages. The effect of thyme extract in reducing fat oxidation was more significant than rosemary extract ($p < 0.05$). The effective function of thyme extract in reducing the fat oxidation of sausages was due to the high amount of total phenolic compounds (224.8 mg Gallic acid/g) and antioxidant properties (677.9 μg/ml). Erkan et al. (2008) reported that phenolic compounds such as thymol and carvacrol have the greatest role in the antioxidant properties of essential oils, and the amount of thymol and carvacrol in thyme extract is higher than rosemary extract. The amount of TBARS in the sausage sample packaged in casings loaded with 6% thyme extract was 1.01 (mg malondialdehyde/kg). Labarrere et al. (2011) stated that the TBARS limit for meat products is 1 (mg malondialdehyde/kg). Ribeiro-Santos et al. (2018) has prepared active films based on whey protein with antioxidant properties in salami. They used rosemary oil and cinnamon extract to decrease the amount of TBARS. The films incorporated with essential oil significantly reduced the oxidation status. In the same study, Hugo et al. were conducted to investigate the efficacy of natural antioxidant combinations (0.5% rosemary extract combined with buffered vinegar, and 0.2% rosemary extract combined with green tea) in prolonging the shelf life of US pork sausage relative to that of synthetic antioxidants (Hugo & Hugo, 2015). It has been concluded that the combination of rosemary extract and buffered vinegar was provided the longest shelf life to pork sausages, followed by the combination of rosemary extract and green tea in terms of oxidation prevention. Barbosa-Pereira et al. (2014) investigated the rosemary extract-coated active films in contact with meat samples on the lipid oxidation. Acive packaging let to a reduction of approximately 50% in the TBARS value. Fasseas et al. (2008) has investigated the antioxidant activity of meat treated (homogenized) with oregano and sage essential oils, during meat storage. The results showed that the essential oil treatments significantly reduced the oxidation.

3.4. *Film Color*

Fibrous casings color loaded with the different concentrations of thyme and rosemary extract are shown in Table 3. They presented high L* value and had a yellowish color as indicated by the b* values. However, only casings with the different loading concentrations of thyme and rosemary extract were significantly more yellow than the control films ($p < 0.05$). These casings also presented the highest color change as measured by ΔE values. As mentioned by Pires et al. (2013), the color of a film may influence the acceptability of a product by consumers.

3.5. *Sausage color*

Table 4 shows the color changes of the sausage during the storage period. The color of sausages did not change significantly during storage time ($p > 0.05$). sausage packaging in Rosemary and thyme extract loaded casings had no significant effect on sausages color. Selani et al. (2011) obtain similar results regarding the lack of color change in frozen cooked chicken meat coated with Wine industry residues extracts as natural antioxidants. Teruel et al. (2015) investigated the effect of rosemary extract color on frozen chicken nuggets. This result realized that the color of the extracts has not interfered with the characteristic frozen chicken nuggets color and therefore the used can be commercially applied in such products.

Table 5. Sensory evaluation of sausage packaged with different fibrous casings loaded with thyme and rosemary extract.

Casing	Odor (Storage time(day))					Color (Storage time(day))				
Thyme extract (%)	0	15	30	45	60	0	15	30	45	60
0	3.6±0.48^{Aa}	3.4±0.28^{Aa}	3.4±0.28^{Aa}	3.5±0.28^{Aa}	3.5±0.28^{Aa}	3.5±0.48^{Aa}	3.3±0.48^{Aa}	3.3±0.48^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}
2	3.3±0.42^{Aa}	3.1±0.42^{Aa}	3.1±0.42^{Aa}	3.2±0.42^{Aa}	3.2±0.42^{Aa}	3.4±0.39^{Aa}	3.2±0.26^{Aa}	3.2±0.44^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}
4	3.5±0.53^{Ab}	3.6±0.32^{Ab}	3.6±0.32^{Ab}	3.4±0.32^{Ab}	3.4±0.32^{Ab}	4.2±0.67^{Ab}	4.1±0.38^{Ab}	4.1±0.57^{Ab}	4.0±0.38^{Ab}	4.0±0.38^{Ab}
6	3.5±0.48^{Ac}	3.5±0.48^{Ac}	3.5±0.48^{Ac}	3.6±0.48^{Ac}	3.6±0.48^{Ac}	4.6±0.48^{Ac}	4.6±0.64^{Ac}	4.6±0.48^{Ac}	4.5±0.52^{Ac}	4.5±0.36^{Ac}
Rosemary extract (%)										
0	3.5±0.33^{Aa}	3.6±0.45^{Aa}	3.6±0.43^{Aa}	3.5±0.38^{Aa}	3.5±0.48^{Aa}	3.6±0.31^{Aa}	3.4±0.48^{Aa}	3.4±0.48^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}
2	3.0±0.34^{Ab}	3.2±0.57^{Ab}	3.2±0.52^{Ab}	3.1±0.22^{Ab}	3.1±0.22^{Ab}	4.4±0.28^{Ab}	4.3±0.78^{Ab}	4.3±0.78^{Ab}	4.5±0.62^{Ab}	4.5±0.58^{Ab}
4	3.8±0.66^{Ac}	3.9±0.64^{Ac}	3.9±0.34^{Ac}	3.8±0.44^{Ac}	3.8±0.54^{Ac}	5.3±0.48^{Ac}	5.0±0.34^{Ac}	5.0±0.34^{Ac}	5.1±0.48^{Ac}	5.1±0.48^{Ac}
6	3.5±0.45^{Ad}	3.5±0.42^{Ad}	3.5±0.48^{Ad}	3.3±0.47^{Ad}	3.3±0.48^{Ad}	5.6±0.35^{Ad}	5.4±0.39^{Ad}	5.4±0.39^{Ad}	5.6±0.28^{Ad}	5.6±0.35^{Ad}

Casing	Texture (Storage time(day))					Flavor (Storage time(day))				
Thyme extract (%)	0	15	30	45	60	0	15	30	45	60
0	3.0±0.25^{Aa}	3.1±0.25^{Aa}	3.2±0.25^{Aa}	3.0±0.44^{Aa}	3.0±0.23^{Aa}	3.5±0.44^{Aa}	3.5±0.44^{Aa}	3.5±0.44^{Aa}	3.5±0.44^{Aa}	3.5±0.44^{Aa}
2	3.4±0.34^{Ab}	3.4±0.34^{Ab}	3.6±0.34^{Ab}	3.5±0.34^{Ab}	3.5±0.34^{Ab}	3.6±0.33^{Aa}	3.6±0.33^{Aa}	3.6±0.33^{Aa}	3.6±0.33^{Aa}	3.6±0.33^{Aa}
4	4.6±0.47^{Ac}	4.5±0.47^{Ac}	4.6±0.47^{Ac}	4.4±0.27^{Ac}	4.4±0.47^{Ac}	3.5±0.38^{Aa}	3.5±0.38^{Aa}	3.5±0.38^{Aa}	3.5±0.38^{Aa}	3.5±0.38^{Aa}
6	4.6±0.44^{Ad}	4.5±0.44^{Ad}	4.7±0.44^{Ad}	4.6±0.24^{Ad}	4.6±0.44^{Ad}	3.6±0.58^{Aa}	3.6±0.58^{Aa}	3.6±0.58^{Aa}	3.6±0.58^{Aa}	3.6±0.58^{Aa}
Rosemary extract (%)										
0	3.5±0.44^{Aa}	3.4±0.44^{Aa}	3.3±0.34^{Aa}	3.5±0.34^{Aa}	3.5±0.34^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}	3.5±0.48^{Aa}
2	4.2±0.25^{Ab}	4.1±0.25^{Ab}	4.0±0.25^{Ab}	4.1±0.25^{Ab}	4.1±0.25^{Ab}	3.5±0.68^{Aa}	3.5±0.68^{Aa}	3.5±0.68^{Aa}	3.5±0.68^{Aa}	3.5±0.68^{Aa}
4	5.2±0.51^{Ac}	5.3±0.51^{Ac}	5.2±0.22^{Ac}	5.4±0.22^{Ac}	5.4±0.22^{Ac}	3.6±0.55^{Aa}	3.6±0.55^{Aa}	3.6±0.55^{Aa}	3.6±0.55^{Aa}	3.6±0.55^{Aa}
6	5.7±0.43^{Ad}	5.8±0.43^{Ad}	5.8±0.43^{Ad}	5.9±0.43^{Ad}	5.9±0.43^{Ad}	3.5±0.34^{Aa}	3.5±0.34^{Aa}	3.5±0.34^{Aa}	3.5±0.34^{Aa}	3.5±0.34^{Aa}

Casing	Overall acceptance (Storage time(day))				
Thyme extract (%)	0	15	30	45	60
0	3.3±0.24^{Aa}	3.4±0.29^{Aa}	3.4±0.26^{Aa}	3.5±0.21^{Aa}	3.5±0.29^{Aa}
2	3.7±0.47^{Ab}	3.8±0.42^{Ab}	3.8±0.43^{Ab}	3.9±0.42^{Ab}	3.9±0.42^{Ab}
4	4.3±0.48^{Ac}	4.2±0.44^{Ac}	4.2±0.44^{Ac}	4.4±0.41^{Ac}	4.4±0.41^{Ac}
6	5.0±0.27^{Ad}	5.1±0.14^{Ad}	5.1±0.15^{Ad}	5.0±0.12^{Ad}	5.0±0.12^{Ad}
Rosemary extract (%)					
0	3.2±0.41^{Aa}	3.3±0.48^{Aa}	3.3±0.22^{Aa}	3.5±0.13^{Aa}	3.5±0.44^{Aa}
2	3.0±0.11^{Ab}	3.0±0.32^{Ab}	3.0±0.34^{Ab}	3.1±0.65^{Ab}	3.1±0.79^{Ab}
4	3.1±0.32^{Ac}	3.3±0.63^{Ac}	3.3±0.63^{Ac}	3.3±0.63^{Ac}	3.3±0.63^{Ac}
6	3.0±0.18^{Ad}	3.2±0.22^{Ad}	3.2±0.31^{Ad}	3.0±0.45^{Ad}	3.0±0.49^{Ad}

Results are shown reported as mean ± standard deviation. For each casing in the same column, different superscript letters (a, b, c) means that values are significantly different ($p < 0.05$). For each casing in the same row, different superscript letters (A, B, C) means that values are significantly different ($p < 0.05$).

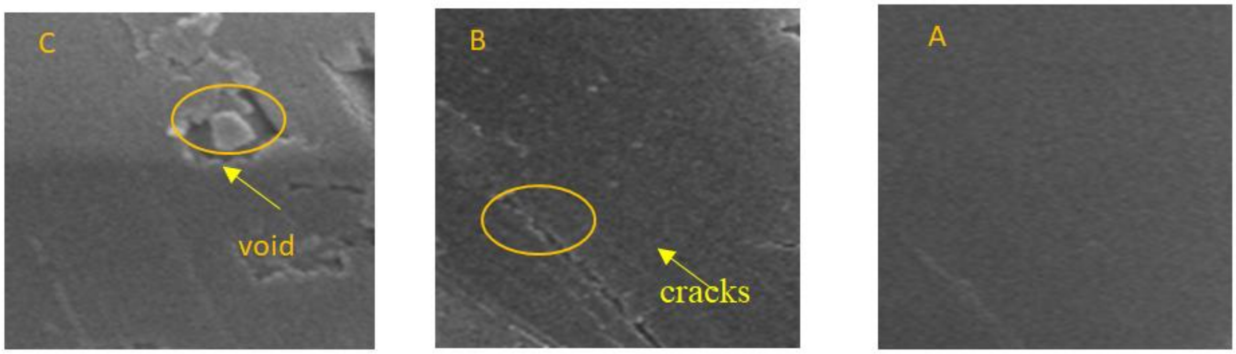

Fig. 3. Surface morphological properties of fibrous casing film without extract (A), loaded with rosemary extract (6%) (B), and loaded with thyme extract (6%) (C).

3.6. *Sensory evaluation*

The sensory analysis of packaged sausages in different types of casings was shown in Table 5. All the scores obtained were ranged between 3.0 and 5.9 and there was a significant ($p < 0.05$) difference between the treatments in any of the parameters measured. Sausages in casing loaded with 2% thyme extract did not show a difference significantly ($p > 0.05$) in terms of odor from the control (casing without extract). However, in the sausages packaged in casing loaded with 4 and 6% thyme extract, as well as all treated casings with different concentrations of rosemary extract the panelists recognized a significant difference. The panelists did not feel a significant difference ($p > 0.05$) in odor characteristics during the storage time. The scores obtained in connection with color assessment were ranged between 3.1 and 3.9. All samples of sausages coated with different levels of thyme and rosemary extract were not significantly different from the control (without coating with extract) sample. Trained panelists did not feel a significant difference ($p > 0.05$) in color characteristics during the maintenance period. As shown in Table 5, there was significant ($p < 0.05$) difference among treatments in flavor. The scores obtained in connection with flavor assessment were ranged between 3.0 and 5.9. Sausage samples coated with 6% thyme extract had a score of 4.6 of moderate flavor intensity. Panelists did not feel a significant difference in sausage texture when testing the 8 treatments. The scores were between 3 and 3.6. There was no significant difference ($p > 0.05$) between treatments in texture. As shown in Table 5, there was a significant ($p < 0.05$) difference among treatments in overall acceptance. The sausage sample coated with 6% thyme extract had the highest overall acceptance score. These results indicate that the sausage sample coated with 6% thyme extract improved the characteristics of sausages and had marked influence on the overall acceptance scores. Generally, it was observed that most of the scores of color, flavor, texture, and overall acceptance were above moderately desirable. Results also indicate that sausage samples coated with 6% thyme extract were preferred by the panelists group and received the highest acceptability scores compared with others while sausages coated with different levels of rosemary extract received the least acceptability scores. Ghabraie et al. (2015) carried out a study to assess the antimicrobial activities of 32 different essential oils against 5 different foodborne pathogens and spoilage bacteria. Further, to select the best combined essential oils with high antimicrobial effects for food application, the combined effects of different essential oils were evaluated, and sensorial analysis was performed to determine the organoleptically acceptable concentration of selected essential oil combination directly added on ground meat as a food model. Sensory evaluation of combined Chinese cinnamon and Cinnamon bark essential oil used in cooked meat was conducted, and an essential oil level of 0.05% was the highest organoleptically acceptable. Quesada et al. (2016) concluded that thyme odor was perceived as desirable in cooked meat and Catarino et al. (2017) have applied WPC active coating containing *Origanum virens* essential oil on the surface of 2 traditional Portuguese sausages during industrial production. The sensory analysis revealed little differences between coated and uncoated sausage attributes, suggesting good acceptance of both.

3.7. *Morphological properties*

The morphological properties of produced fibrous casing films are represented in Fig. 3. Fibrous casing film without extract (A) showed an ordered and homogeneous structure without pores and cracks. While in the active films (B and C) some cracks were observed on the fractured surfaces. Pores and cracks were more evident in the film with 6% of rosemary and thyme extracts. Due to this, the water vapor diffusion was increased, reducing the barrier to water vapor in this film. Piñeros-Hernandez et al. (2017) reported the same structure for edible cassava starch films containing rosemary antioxidant extracts.

4. Conclusion

In this study, fibrous casting loaded with different concentrations of thyme and rosemary extract (0, 2, 4 and 6%) has been designed as a novel active packaging for sausages packaging. Active fibrous casing was evaluated for sausage packaging in terms of vapor permeability, color, oxidation index and sensory evaluation. Addition rosemary and thyme extracts (2-4%) in the fibrous casing increases the water vapor permeability. The color of the fibrous coating changed with increasing the load of rosemary extract and thyme. This increase in color (ΔE) reduces light absorption and reduces oxidation during storage in sausages. The best active fibrous casing with antioxidant properties was fibrous casing coated with 6% thyme extract. Sausage active casings loaded with rosemary extract showed more antioxidative effects compared the casings which loaded with thyme extract and control (without extract), respectively. At the end of the cold storage period, sausages packaged in casing loaded with 6 % thyme extract reduced the amount of TBARS compared to the control sample by 30%. In fact, casing loading with natural antioxidant extract demonstrated that the application of active films can reduce the lipid oxidation of meat products and extending their shelf life while releasing natural antioxidants to their surface. Further studies are demanded, namely the identification of the antioxidant activity of the novel natural materials and their behavior when in direct contact with food matrices.

Acknowledgment

The authors thank the Iranian Research Organization for Science & Technology (IROST) and Research Institute of Forest and Rangelands for technical and laboratory support. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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