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Combined effects of microencapsulated essential oils and irradiation from gamma and X-ray sources on microbiological and physicochemical properties of dry fermented sausages during storage

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ABSTRACT

Essential oils (EOs) or EOs encapsulated in alginate and alginate-cellulose nanocrystal combined with 1.5 kGy X-ray (0.76 kGy/h) or γ-ray (6.37 kGy/h) irradiation were applied on dry fermented sausages (DFS). Microbiological quality was tested in terms of the reduction of *Escherichia coli* O157:H7 cocktail, *Listeria monocytogenes*, molds and yeasts, lactic acid bacteria (LAB), and total mesophilic bacteria (TMF) during storage at room temperature (20 ± 1 °C) for 8 weeks. Physicochemical quality was tested by following the changes of texture and color of sausages. Synergistic effects were observed with combined treatments with γ-irradiation on inhibiting *E. coli* O157:H7 cocktail and LAB and with X-ray on inhibiting *E. coli* O157:H7 cocktail. Extensive inhibition of *L. monocytogenes*, molds and yeasts, and TMF was also noticed during storage. Antimicrobial formulations combined with γ-irradiation did not show adverse effects on texture and color of sausages while treatment with X-ray resulted in the reduction of redness and increase of hardness. However, the differences of texture were eliminated during storage.

1. Introduction

Food irradiation has been historically in use for more than 100 years and is increasingly being accepted and widely recognized as a part of overall good manufacturing practice (GMP) and hazard analysis critical control points (HACCP) systems (Diehl, 2002; Shah, Mir, & Pala, 2021). Food irradiation is a process of exposing food to the controlled amounts of ionizing radiations such as γ-rays, X-rays and accelerated electrons, to reduce food-borne pathogens, spoilage microorganisms and parasites, extend shelf-life, disinfect insects, detoxify toxic substances and maintain nutrition (Indiarto, Pratama, Sari, & Theodora, 2020; Singh & Singh, 2020). It is a non-thermal method that can retain the sensory properties and product qualities (Pedreschi & Mariotti-Celis, 2020). Therefore, irradiation is particularly useful for the decontamination of foods that are sold without thermal treatments such as raw poultry, meat, and seafood (Shah et al., 2021). γ-ray and X-ray are short wavelength radiations with very high associated energy levels (Lacroix, 2014). Cobalt-60 is the most commonly used radionuclide for food in the form of γ rays. X-rays that have high penetrating power and no left radiation hazards are raising interests as low risk-significant radioactive sources (Indiarto & Qonit, 2020). However, few studies have been done in use of X-ray for food pasteurization (Begum et al., 2020). The mechanism of ionizing radiation is mainly related to the damage of nucleic acids, interruption of chemical bonds in DNA, which directly or indirectly caused by oxidative free radicals generated from water radiation decomposition (Lacroix, 2014).

Essential oils (EOs) are aromatic volatile oily liquids extracted from plant materials, which generally recognized as safe (GRAS) as food additives (Burt, 2004). Due to different biosynthetic chains, EOs are a complex mixture of natural compounds divided into the terpene group and the aromatic and aliphatic group (Falleh, Ben Jemaa, Saada, & Ksouri, 2020). The main constituents of EOs are phenolic compounds which are one of the most important molecules determining the biological properties of EOs (Varghese, Siengchin, & Parameswaranpillai, 2020).

The antimicrobial activity of EOs has been widely recognized by previous researches against foodborne pathogens and food spoilage fungi (Faleiro, 2011; Ji, Shankar, Royon, Salmieri, & Lacroix, 2021). Among the EOs, the good antimicrobial activity of Cinnamon EO and its potential utilization in preservation of food has been reported in

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literatures (Clemente, Aznar, & Nerín, 2019; Oussalah, Caillet, & Lacroix, 2006). Cinnamon EO showed antimicrobial activity against *E. coli* O157:H7, *L. monocytogenes*, *L. innocua*, *Salmonella* Typhimurium, and *Salmonella enterica* (Ji, Shankar, Fernandez, et al., 2021). Accumulation of antimicrobial compounds on the membrane results in fluidifying effect on the membrane, leakage of intracellular constituents and cell death through various mechanisms depending on different components of EOs and target microorganisms (Ji, Shankar, Royon, et al., 2021; Pateiro et al., 2021). The use of EOs or their constituents as food preservatives is often limited due to a required higher concentration in food models causing negative organoleptic effects. In addition, food components such as fat, starch, protein can interact with hydrophobic compounds that reduce the antimicrobial activity of EOs (Hyldgaard, Mygind, & Meyer, 2012). Applying a combination of various EOs in the form of encapsulation is a good alternative with increasing antimicrobial activity without increasing the concentration of EOs (Calo, Crandall, O'Bryan, & Ricke, 2015). Encapsulating EOs into biopolymers as films, coatings or sachets protects the bioactivity of EOs during the processing and storage, controlling their release while avoid the intense aroma of EOs (Castro-Rosas et al., 2017).

Alginate is a polysaccharide extracted from brown algae. Alginate biopolymer has been widely used in many fields such as food packaging, drug delivery, tissue engineering and wound dressing, which is due to its biocompatibility, nontoxicity, low cost, and easily gelation properties (Huq, Riedl, Bouchard, Salmieri, & Lacroix, 2014). Microencapsulation of bioactive compounds in alginate can be a promising way to control their release in food matrix. However, some drawbacks of alginate such as hydrophilic behavior and low stability can limit its application. For overcoming this limitation, incorporation of cellulose nanocrystal (CNC) as a nano-sized material in biopolymer in order to improve the physicochemical properties of biopolymer and also create tortuous pathways in its matrices, have been used. Besides, the release of encapsulated active compounds from the alginate-CNC can be controlled (Criado et al., 2019).

Dry fermented sausages (DFS) are defined as sausages that have a final pH ranging from 5.2 to 5.8, the moisture lower than 30%, a_w from 0.85 to 0.91, and moisture:protein ratio lower than 2.3:1 (Vignolo, Fontana, & Fadda, 2010). The production of DFS consists of three clearly-defined steps: ingredients mixing, fermentation and drying (Fernández-López, Sendra, Sayas-Barberá, Navarro, & Pérez-Alvarez, 2008). During the three steps, the physical, chemical and microbiological diversifications are closely related to the raw material characteristics and the process conditions (Fernández-López et al., 2008; Houben & van't Hooft, 2005). Because of the relative high level of fat and distinctive processing characteristics such as the use of diverse raw materials, absence of thermal treatment, fermented sausages are highly subjected to quality deterioration, which mainly includes lipid oxidation and microbial deterioration (Tomović et al., 2020). Many studies have reported that some pathogens like *L. monocytogenes*, *Salmonella* and *E. coli,* can survive in DFS and cause many cases of foodborne disease outbreaks in many countries (Lindqvist & Lindblad, 2009).

The aim of this study was application of free EOs and encapsulated EOs in alginate or alginate-CNC combined with 1.5 kGy γ-rays (6.37 kGy/h) and X-rays (0.76 kGy/h) in DFS to compare the effects of γ-ray at high dose rate with X-ray at low dose rate on microbial and physicochemical properties of DFS during storage. The results are expected to provide a useful reference for reasonable application of two types of ionizing radiation in combination with EOs, in form of free and encapsulated on DFS.

2. Materials and methods

2.1. Bacterial culture preparation

Before tests, cultures from stock at − 80 °C were propagated through 3 successive growth cycles at 37 °C for 24 h in Tryptic Soy Broth (TSB; Becton-Dickinson, Sparks, MD, USA) for *E. coli* cocktail (mixture of five *E. coli* O157:H7 strains of RM1239, RM1931, RM1933, RM1934, 380-94) and *L. monocytogenes* (LM 1045) to obtain a concentration of approximately 10^{12} CFU mL^{-1} and 10^{9} CFU mL^{-1} respectively.

2.2. Antimicrobial formulations

The formulations were prepared based on the method of Huq, Vu, Riedl, Bouchard, and Lacroix (2015) with some modifications. A 2% (w/v) of alginate (Sigma-Aldrich Co., Oakville, ON, Canada) and a 1% (w/v) CNC (CelluForce Inc. Montreal, QC, Canada) suspension were prepared in deionized water under magnetic stirring for 24 h. 1000 J/g ultra-sonication (QSonica Q-500 sonicator, Fisher Scientific, Ottawa, ON, Canada) was then applied on CNC suspension. A 5% (w/w) CNC-alginate suspension was homogenized for 2 min at 20,000 rpm using an IKA Ultra-Turrax T 25 digital disperser (IKA Works Inc., Wilmington, NC, USA). An emulsified Cinnamon EOs (3%) with Tween 80 (5% w/v, Sigma–Aldrich Co.) was added to alginate and alginate–CNC suspensions and mixed well, then a solution of 0.01 M $CaCl_2$ (Sigma-Aldrich Co.) was added dropwise with a ratio of 75:25 to alginate-EO and alginate–CNC–EO emulsion. The final concentration of encapsulated EOs was 2.25%. Four (4) formulations were prepared following the procedure (Ji, Shankar, Salmieri, & Lacroix, 2022) including non-encapsulation (EO), alginate encapsulated EO (AE), alginate-CNC encapsulated (ACE) and non-EOs as a control (CT).

2.3. Sausage manufacture

Manufacturing protocol and materials (beef of 23% fat, 19.7% of protein, spices, casing, and ferments) were provided by Usine Amsellem (Laval, QC, Canada). The sausage was prepared based on method of Ji et al. (2022) with some modifications. The 4% *E. coli* cocktail and 0.1% *L. monocytogenes* were inoculated to ground meat to obtain around 7.5 log and 5 log separately before manufacturing. Three (3) prepared formulas were mixed to meat to obtain a final concentration of 0.45% EO before casing. 50-g sausages were cased by Tre Spade sausage stuffing (Mod. 10 Deluxe; P/N 21100/L; FACEM SpA, Turin, Italia). Final products were obtained after a 48-h fermentation (25 ± 0.3 °C, 90 ± 2% RH) with an ending pH of about 5.20, and a 5-d drying (141 °C, 70 5% RH) with an ending a_w of about 0.85. Sausages were then vacuum packed by using a Sipromac vacuum packaging machine (model 350; Sipromac, Drummondville, QC, Canada) and stored at room temperature (20 ± 1 °C).

2.4. Sample irradiation

The γ-irradiation procedure was done at the Canadian Irradiation Centre (CIC, Laval, QC, Canada) in a cobalt-60 Underwater Calibrator UC-15A (energy level: 1.25 MeV; Nordion Inc., Ottawa, ON, Canada) at a dose rate of 6.37 kGy/h. X-ray irradiation was realized in INRS-Armand Frappier Health Biotechnology Research Centre (Laval, QC, Canada) using a Philips MG160 X-ray machine (125 keV; 16 mA) at a dose rate of 0.76 kGy/h. Samples were irradiated to 1.5 kGy by X- or γ-ray. Four (4) groups of CT, EO, AE, ACE treated with γ-irradiation were CT + GI, EO + GI, AE + GI, ACE + GI. Four (4) groups treated with X-ray irradiation were CT + XI, EO + XI, AE + XI, ACE + XI.

2.5. Microbiological analysis

Each 10 g sausage sample was mixed in 90 mL of peptone water (0.1%) and homogenized at 260 rpm for 1 min in a Seward 400 Circulator Stomacher® (Fisher Scientific). Tryptic soy agar (TSA), De Man, Rogosa and Sharpe (MRS) agar, and potato dextrose agar (PDA) were from Alpha Biosciences (Baltimore, MD, USA) and were used for total mesophilic flora (TMF; 37 ± 1 °C, 48 h), lactic acid bacteria (LAB; 30 ± 1 °C, 72 h), and yeasts and molds (Y/M; 25 ± 1 °C, 72 h), separately.

Palcam Agar supplemented with antibiotics acriflavine (5 mg/mL), polymyxin B (10 mg/mL), and ceftazidime (8 mg/mL) and MacConkey Sorbitol Agar (Oxoid Ltd. Nepean, Ottawa, ON, Canada) were used for *L. monocytogenes* (37 ± 1 °C °C, 48 h) and *E. coli* O157:H7 (37 ± 1 °C, 24 h), respectively. Sampling was performed at the end of drying before irradiation and after irradiation, at 4th week and 8th week during storage. The detection limit was 10 CFU/g.

2.6. Color

Color was measured using a Konica Minolta Color reader CR10-Plus (Konica Minolta Inc., Ramsey, NJ, USA) (Ben Fadhel et al., 2016). The outer color was measured on the surface of sausages; the inner color was measured on the tangent plane. Color was expressed in CIE-LAB system, L^* for lightness, a^* for redness and b^* for yellowness. The total change of color ΔE* was calculated according to Equation (1).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

2.7. Texture

Texture was measured according the method of Houben and van't Hooft (2005) with modifications. Samples were cut into 2 cm thick slices with a flat tangent plane. Tests were performed at room temperature with a Universal Testing Machine (UTM model H5KT, Tinius-Olsen Testing Machine Co., Inc., Horsham, PA, USA) equipped with a 100 N-load cell (type FBB) and 1.5 kN specimen compression platens. A "Compression from position" test type was set up and compression was performed up to 20% of the original portion height with an aluminium cylinder probe of 1 cm diameter. Force-time deformation curves were obtained by fixing the position rate of machine control at a cross-head speed of 20 mm/min. Stress-strain curves were determined from force-distance recordings. Maximum stress and Young's modulus (maximum slope of the stress-strain curve between the origin and the yield point) were recorded indicating the hardness of samples by using Test Navigator program ver. 7.02.11.

2.8. Statistical analysis

For all results, a one-way analysis of variance (ANOVA), Tamhane's test for unequal variances and Duncan's multiple-range test for equal variances were performed by PASW Statistics 18 software (IBM Corporation, Somers, NY, USA). Differences between means were considered significant when the confidence interval was lower than 5% ($P \leq 0.05$). Experiments were done in triplicate (n = 3). For each replicate, 2 samples from each treatment group were analyzed for microbial tests and 3 samples were analyzed for color and texture during storage.

Table 1
Reduction of microbial counts during storage at room temperature[1].

	Concentration of *E. coli* cocktail (log CFU/g)			Concentration of *L. monocytogenes* (log CFU/g)			Concentration of Y/M (log CFU/g)			Concentration of LAB (log CFU/g)			Concentration of TMF (log CFU/g)		
Samples	After drying	4weeks	8weeks	After drying	4weeks	8weeks	After drying	4weeks	8weeks	After drying	4weeks	8weeks	After drying	4weeks	8weeks
CT	4.84 ± 0.09 Cc	2.63 ± 0.32 Bb	ND Aa	3.94 ± 0.69 Cb	ND Aa	ND Aa	6.87 ± 0.48 Cc	4.00 ± 0.54 Bb	ND Aa	8.94 ± 0.31 Gc	7.62 ± 0.31 Fb	6.61 ± 0.18 Ea	9.28 ± 0.28 Fc	8.64 ± 0.12 Gb	8.30 ± 0.30 Da
EO	4.13 ± 0.75 Cb	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	8.34 ± 0.18 Fc	7.53 ± 0.39 Fb	4.99 ± 0.13 CDa	8.33 ± 0.27 Ec	7.66 ± 0.39 Fb	7.10 ± 0.70 Ca
AE	4.44 ± 0.33 Cb	ND Aa	ND Aa	1.26 ± 0.45 Bb	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	8.29 ± 0.28 Fc	7.54 ± 0.14 Fb	5.12 ± 0.26 Da	8.48 ± 0.25 Ec	7.73 ± 0.27 Fb	7.02 ± 0.84 Ca
ACE	4.32 ± 0.24 Cb	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	8.19 ± 0.12 Fc	7.37 ± 0.50 Fb	5.32 ± 0.59 Da	8.32 ± 0.17 Ec	7.59 ± 0.14 Fb	6.93 ± 0.64 Ca
CT + GI	4.06 ± 0.31 Cc	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	5.66 ± 0.31 Bb	1.62 ± 0.87 Aa	ND Aa	7.13 ± 0.29 DEb	4.65 ± 0.13 Ca	4.50 ± 0.69 BCa	7.37 ± 0.31 Dc	6.04 ± 0.17 DEb	5.50 ± 0.18 Ba
EO + GI	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	5.36 ± 0.22 Ab	3.74 ± 0.50 Ba	3.72 ± 0.42 Aa	6.23 ± 0.15 Ac	5.42 ± 0.23 ABb	4.93 ± 0.08 Ba
AE + GI	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	5.72 ± 0.27 Bb	3.72 ± 0.62 Ba	3.76 ± 0.20 Aa	6.65 ± 0.16 BCc	5.93 ± 0.09 CDEb	4.99 ± 0.26 Ba
ACE + GI	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	5.26 ± 0.31 Ab	3.13 ± 0.18 Aa	3.29 ± 0.02 Aa	6.36 ± 0.06 ABc	5.22 ± 0.20 Ab	4.91 ± 0.13 Ba
CT + XI	4.62 ± 0.06 Cc	2.53 ± 0.35 Bb	ND Aa	ND Aa	ND Aa	ND Aa	5.38 ± 0.10 Bc	1.30 ± 0.00 Ab	ND Aa	7.21 ± 0.22 Ec	6.40 ± 0.12 Eb	4.71 ± 0.22 CDa	7.19 ± 0.25 Dc	6.18 ± 0.13 Eb	5.78 ± 0.29 Ba
EO + XI	2.45 ± 0.13 Bb	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	6.71 ± 0.10 Cc	5.64 ± 0.02 Db	3.35 ± 0.07 Aa	6.71 ± 0.38 Cc	5.57 ± 0.11 ABCb	3.20 ± 0.12 Aa
AE + XI	2.63 ± 0.30 Bb	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	6.95 ± 0.38 CDEc	5.44 ± 0.29 Db	3.94 ± 0.21 ABa	6.7 ± 0.47 Cc	5.70 ± 0.00 BCDb	3.85 ± 0.21 Aa
ACE + XI	2.56 ± 0.49 Ba	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	ND Aa	6.87 ± 0.09 CDc	5.70 ± 0.05 Db	3.68 ± 0.03 Aa	6.74 ± 0.18 Cc	5.68 ± 0.15 BCDb	3.33 ± 0.35 Aa

[1]Numbers are means ± standard deviations from triplicate samples.Within each row for each tested strain, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column, means with the same uppercase letter are not significantly different ($P > 0.05$).

3. Results and discussion

3.1. E. coli elimination

The reduction of the tested microorganisms during the whole process is shown in Table 1. The treatments of EO, AE, and ACE showed no difference in *E. coli* count at the end of drying ($p > 0.05$) but contributed similarly to eliminate *E. coli* at the 4th week of storage while the control group had 2.63 log CFU/g *E. coli* after 4 weeks of storage. **γ**-irradiation showed no significant effect at the end of drying when applied individually but reduced *E. coli* from 4.06 log CFU/g to not-detectable level at 4th week compared to the control. X-ray irradiation in CT + XI group also showed no significant effect at the end of drying but a 2.09-log reduction was observed within 4 weeks of storage. The reduction amount was comparable with the control group without irradiation indicating that *E. coli* is more resistant to X-ray than γ-ray. Synergistic effects were observed when γ-irradiation was used in combination with the free EOs, AE, and ACE. The combined treatments of EO + GI, AE + GI and ACE + GI eliminated *E. coli* at the end of drying. But the synergistic effect of both free EOs and encapsulated ones when combined with X-ray irradiation was not as great as the similar treatments with γ-irradiation at the end of drying so that *E. coli* was around 2.5 log CFU/g and was not eliminated as what happened in γ-irradiation treatments.

Previous researches have demonstrated the inhibitory effects of EOs and irradiation on *E. coli*. Sage EO applied in minced pork significantly reduced the growth of *E.coli* during storage (Danilović et al., 2021). Edible coatings based on agar/sodium alginate containing ginger EO applied on sliced fresh beef were proved to extend shelf-life well by inhibiting *E. coli*, Y/M and total viable counts during refrigerated storage (Zhang et al., 2021). Cho and Ha (2019) studied the effects of X-ray for the inactivation of foodborne pathogens in ready-to-eat sliced ham. *E. coli* O157:H7 was eliminated to a not-detectable level when irradiated at ≥ 0.6 kGy. Begum et al. (2020) tested γ- and X-ray sources at different dose rates combined with oregano/thyme EO against *E. coli* O157:H7 in rice. The radiosensitivity of bacterium was significantly affected by different dose rates and various EOs, also combined treatment had the synergistic inhibitory impact on *E. coli*. *E. coli* O157:H7 was observed to be more resistant to X-ray than γ-ray in rice due to the lower dose rates which are in agreement with our test.

3.2. L. monocytogenes elimination

The samples treated with free EOs, ACE and all irradiated with γ- and X-ray were devoid of *L. monocytogenes* at the end of drying (Table 1). But the treatment of AE reduced *L. monocytogenes* by 2.68 log CFU/g after drying compared to control, and were able to eliminate *L. monocytogenes* at the 4th week of storage.

In this test, *L. monocytogenes* was eliminated to below detection level with free EOs or the ACE treatment without irradiation. The radiosensitivity of *L. monocytogenes* to X-ray or γ-irradiation cannot be compared in this study since the remaining bacteria in both treatments are below detection limit. However, it has been already observed that *L. monocytogenes* is more resistant to X-ray and γ-irradiation than *E. coli* O157:H7 (Cho & Ha, 2019; Tawema, Han, Vu, Salmieri, & Lacroix, 2016). According to Begum et al. (2020), *L. monocytogenes* was found to have higher D_{10} values with X-ray at dose rate of 0.76 kGy/h than γ-ray treatments applied at dose rates of 9.1, 3.93 and 0.22 kGy/h and synergistic effects were observed with the combination of X-ray/γ-ray irradiation with oregano/thyme EOs. It has been also reported the synergistic effects of 1.5 kGy γ-irradiation with oregano or cinnamon EO and nisin microencapsulated in alginate-CNC against *L. monocytogenes* in ready-to-eat ham (Huq et al., 2015).

3.3. Y/M elimination

The EOs with or without encapsulation eliminated Y/M upon completing drying storage (Table 1). X-ray and γ irradiation reduced significantly Y/M counts around 1.49 log and 1.21 log CFU/g respectively, at the end of drying. The more reduction was observed at 4th week of storage, with respective values by 2.7 log and 2.38 log CFU/g for X-ray and γ-ray treatments as compared to the control. Therefore, X-ray and γ-ray have similar effects on reducing the Y/M population when used irradiation alone. The findings of previous researchers are not in accordance with our results. However, Y/M were more resistance to X-ray and γ-ray irradiation than *L. monocytogenes* and *E. coli* O157:H7 cocktail in this study. The use of EOs led to increase in the sensitivity of Y/M, which implies a higher susceptibility to the applied EOs than irradiation. The findings of previous research are not in accordance with our results. *A. niger* showed more resistance to X-ray at 0.76 kGy/h than γ-ray at 0.085, 4.558 and 10.445 kGy/h (Shankar et al., 2020). However, when X-ray (0.76 kGy/h) was combined with EOs, the lowest radio sensitivity was observed for *A. niger* similar to γ-ray (10.445 kGy/h) combined with EOs.

3.4. LAB elimination

According to the results (Table 1), although applying EOs reduced the LAB in the product, the encapsulation had no tangible effect on the LAB compared to free EOs. X-ray and γ-ray reduced LAB approximately by 1.73 and 1.81 log CFU/g, respectively, at the end of drying, which demonstrates the similar effects of X-ray and γ-ray in deactivation of LAB. However, their effectiveness became different during storage after 4 weeks. X-ray and γ-ray reduced LAB by 1.22 and 2.97 log CFU/g, respectively, compared to control. Samples treated with X-ray had significantly higher count in LAB, which shows that LAB was more resistant to X-ray than γ-ray. After 8 weeks, the groups treated with X-ray and γ-ray showed similar LAB loss. A synergistic effect was observed when γ-irradiation combined with free EOs and their encapsulated forms (ACE and AE) after drying and during 4 weeks of storage. At 8th week of storage, following total release of active compounds in the product the similar antimicrobial activity was observed for EOs, AE, and ACE. The more efficient role of γ-irradiation in comparison with X-ray in reducing LAB was proved in the combined treatments. Regarding X-ray, the presence of EOs increased the radiosensitivity of LAB, but the type of encapsulation material had no effect on this parameter during the entire storage period.

The resistance of LAB during storage and its presence as dominant microflora were also demonstrated in vacuum packed sausages by Rubio et al. (2007). Combined effect of active chitosan-based films containing cumin EO nanoemulsion and 2.5 kGy γ-irradiation was observed to reduce significantly total mesophilic bacteria and LAB and extend shelf-life of beef loins during chilled storage (Dini, Fallah, Bonyadian, Abbasvali, & Soleimani, 2020). Gelatin-CMC films incorporated with chitin nanofiber and higher concentration of *Trachyspermum ammi* EO (1%) reduced most total viable counts, LAB and molds and yeasts in raw beef (Azarifar, Ghanbarzadeh, Sowti khiabani, Akhondzadeh basti, & Abdulkhani, 2020).

3.5. TMF elimination

The decrease in the count of TMF during the storage of irradiated meat is due to the post-irradiation effect, in a way that the surviving cells damaged by γ-rays cannot adapt to the surrounding environment and gradually die (Kim et al., 2000). The impact of free EOs was similar to that of their encapsulated forms and the reduction value was approximately 1 log CFU/g after drying. It is clear that the storage time had pronounced decreasing effect for the treatments of EOs, AE, and ACE. X-ray and γ-ray reduced by 1.91 and 2.09 log CFU/g of TMF, respectively, at the beginning of storage and these values increased to 2.46 and 2.6 log CFU/g, respectively, at the 4th week of storage and to 2.52 log and 2.8 log CFU/g at 8th week after drying. Similar trends of X-ray and γ-ray in combination with EO, whether free or encapsulated, were

observed for TMF at the end of drying and 4th week of storage but more reduction was observed for combined treatments of X-ray over those of γ-irradiation at 8th week of storage. No synergistic effect was observed for X-ray and γ-ray combined with EO, AE and ACE at the beginning of drying and at the 4th week of storage after drying. However, the synergistic effect was observed only for X-ray in combination with EO, AE, and ACE at 8th week.

Ionizing radicals originated from the radiolysis of water, can damage to cell by destroying the structure and function of cellular components such as DNA, pigments, fatty acids, and membrane lipids therefore causing chromosomal abnormalities, errors in cell division, and inactivation of endogenous enzymes (Ahn, Kim, & Lee, 2013; Cho & Ha, 2019; Kim et al., 2018). Thus, irradiation may enhance the contact between antimicrobial molecules and cell membranes, increasing the inhibitory effects of EOs and radiosensitivity of microorganisms (Fallah et al., 2021; Turgis, Han, Caillet, & Lacroix, 2009; Turgis, Vu, Dupont, & Lacroix, 2012). In the present study, X-ray irradiation reduced TMF by around 2 log CFU/g compared to control, which is in a good agreement with the results observed in previous research investigated the effect of X-ray on beef (Kim et al., 2018).

3.6. Color evaluation of sausages

In all treated and untreated samples, storage time had no significant impact on the L^* parameter, either in the external or internal part of sausages. It is clear from Table 2 that all treatments increased the color coordinate of L^* significantly whether on the surface or within the sausages. Apparently, the changes resulting from γ-irradiation were more tangible than X-ray as compared to the control. The introduction of EO into the product raised the exterior lightness but its encapsulation with polymers created more notable increment in the surface lightness. By comparing the lightness of EO, AE, and ACE formulated samples treated with X- and γ-ray, it seems that the increase of lightness resulted from the presence of EOs but not irradiation, as no significant difference was observed between the L^* values of EO, AE, and ACE formulated samples and respective values to the same samples treated with X- and

Table 2
Color attributes of outside and inside of sausages during storage at room temperature[1].

Samples		Exterior color			Interior color		
		After drying	4weeks	8weeks	After drying	4weeks	8weeks
CT	L^*	38.03 ± 0.55 ^{Aa}	37.33 ± 1.53 ^{Aa}	39.98 ± 0.29 ^{ABb}	37.80 ± 3.49 ^{Aa}	32.67 ± 1.14 ^{Aa}	35.90 ± 0.36 ^{Ba}
	a^*	−4.90 ± 0.78 ^{BCa}	−4.67 ± 0.76 ^{DEa}	−4.18 ± 0.36 ^{DEFa}	−1.50 ± 0.26 ^{CDEa}	−0.83 ± 0.15 ^{Fa}	−0.90 ± 0.10 ^{Ea}
	b^*	1.50 ± 0.36 ^{Aa}	1.47 ± 0.14 ^{ABa}	1.82 ± 0.04 ^{Aa}	3.93 ± 0.67 ^{Aa}	4.00 ± 0.10 ^{Ca}	4.50 ± 0.10 ^{ABa}
	ΔE	–	1.70 ± 0.51 ^{CDE}	2.13 ± 0.18 ^{BC}	–	5.18 ± 1.14 ^{D}	2.08 ± 0.33 ^{AB}
EO	L^*	38.65 ± 0.42 ^{ABDa}	40.13 ± 0.4 ^{CDEb}	40.25 ± 0.06 ^{ABb}	39.50 ± 3.41 ^{Aa}	37.50 ± 0.71 ^{BCa}	38.13 ± 0.12 ^{CDa}
	a^*	−5.68 ± 0.66 ^{Ba}	−5.40 ± 0.56 ^{CDa}	−4.93 ± 0.10 ^{BCa}	−2.03 ± 0.40 ^{BCDa}	−0.38 ± 0.09 ^{FGc}	−1.33 ± 0.12 ^{CDb}
	b^*	1.45 ± 0.37 ^{Aa}	2.07 ± 0.55 ^{BCDa}	2.63 ± 0.30 ^{BCa}	4.73 ± 0.40 ^{ABa}	5.25 ± 0.57 ^{EFa}	5.00 ± 0.10 ^{BCa}
	ΔE	–	1.78 ± 0.09 ^{DE}	2.13 ± 0.21 ^{BC}	–	2.74 ± 0.42 ^{A}	1.57 ± 0.07 ^{AB}
AE	L^*	40.45 ± 0.67 ^{DEa}	40.55 ± 0.44 ^{DEFGa}	41.25 ± 0.37 ^{BCDa}	42.63 ± 4.39 ^{Aa}	40.70 ± 0.58 ^{Ea}	41.87 ± 0.06 ^{Fa}
	a^*	−4.35 ± 0.94 ^{Ca}	−4.70 ± 0.41 ^{DEa}	−4.12 ± 0.46 ^{DEFa}	1.90 ± 0.42 ^{Fc}	0.86 ± 0.21 ^{HIb}	0.20 ± 0.00 ^{Ga}
	b^*	2.93 ± 0.57 ^{Ca}	2.95 ± 0.10 ^{DEa}	3.02 ± 0.64 ^{CDa}	7.30 ± 0.44 ^{Da}	7.16 ± 0.32 ^{Ga}	7.10 ± 0.10 ^{EFa}
	ΔE	–	0.63 ± 0.15 ^{A}	1.12 ± 0.30 ^{A}	–	2.23 ± 0.56 ^{A}	1.88 ± 0.03 ^{AB}
ACE	L^*	40.03 ± 0.78 ^{CDEa}	41.13 ± 0.67 ^{EFGa}	42.3 ± 0.26 ^{DEb}	41.65 ± 2.39 ^{Aa}	37.40 ± 1.32 ^{BCa}	41.33 ± 0.06 ^{EFa}
	a^*	−4.03 ± 0.74 ^{Ca}	−3.45 ± 0.43 ^{Fa}	−4.17 ± 0.45 ^{DEFa}	1.63 ± 0.44 ^{Fc}	0.23 ± 0.06 ^{GHb}	−0.53 ± 0.06 ^{Fa}
	b^*	2.95 ± 0.49 ^{Ca}	4.08 ± 0.14 ^{Fb}	3.80 ± 0.17 ^{Eb}	7.35 ± 1.09 ^{Da}	5.87 ± 0.06 ^{Fa}	6.73 ± 0.21 ^{EFa}
	ΔE	–	1.78 ± 0.38 ^{DE}	2.46 ± 0.22 ^{C}	–	4.74 ± 1.14 ^{CD}	2.27 ± 0.06 ^{BC}
CT + GI	L^*	38.97 ± 0.23 ^{ABCa}	39.28 ± 0.68 ^{BCa}	40.17 ± 0.45 ^{ABa}	40.63 ± 2.68 ^{Aa}	37.20 ± 0.92 ^{BCa}	37.43 ± 0.54 ^{BCa}
	a^*	−5.53 ± 0.55 ^{Ba}	−4.70 ± 0.86 ^{DEa}	−4.07 ± 0.12 ^{DEFa}	−2.93 ± 0.55 ^{Ba}	−2.40 ± 0.35 ^{Da}	−1.55 ± 0.17 ^{Cb}
	b^*	1.30 ± 0.20 ^{Aa}	2.23 ± 0.56 ^{BCDa}	2.17 ± 0.32 ^{ABa}	3.70 ± 0.72 ^{Aa}	3.73 ± 0.64 ^{Ca}	3.80 ± 0.39 ^{Aa}
	ΔE	–	1.62 ± 0.47 ^{CDE}	2.13 ± 0.14 ^{BC}	–	3.54 ± 0.83 ^{ABC}	3.56 ± 0.50 ^{E}
EO + GI	L^*	39.63 ± 0.65 ^{BCa}	40.30 ± 0.17 ^{CDEFa}	40.50 ± 0.08 ^{ABCa}	37.03 ± 2.38 ^{Aa}	39.03 ± 0.12 ^{CDEa}	36.48 ± 0.99 ^{BCa}
	a^*	−6.53 ± 0.40 ^{Aa}	−5.27 ± 0.15 ^{CDb}	−5.25 ± 0.06 ^{Bb}	−1.85 ± 0.07 ^{CDEa}	−1.63 ± 0.31 ^{Ea}	−1.00 ± 0.10 ^{DEb}
	b^*	1.33 ± 0.25 ^{Aa}	2.43 ± 0.31 ^{CDEb}	1.55 ± 0.13 ^{Aa}	4.00 ± 0.35 ^{Aa}	4.93 ± 0.42 ^{DEa}	5.02 ± 0.50 ^{BCa}
	ΔE	–	1.82 ± 0.20 ^{DE}	1.63 ± 0.05 ^{AB}	–	2.25 ± 0.30 ^{A}	1.71 ± 0.47 ^{AB}
AE + GI	L^*	41.02 ± 1.04 ^{EFa}	40.63 ± 0.71 ^{DEFGa}	41.77 ± 0.81 ^{CDEa}	42.33 ± 1.05 ^{Aa}	40.00 ± 1.47 ^{DEa}	40.10 ± 0.20 ^{EFa}
	a^*	−5.65 ± 0.63 ^{Ba}	−4.43 ± 0.36 ^{Eb}	−3.98 ± 0.40 ^{DEFb}	−1.00 ± 0.00 ^{Ea}	1.20 ± 0.18 ^{Ic}	−0.10 ± 0.00 ^{Gb}
	b^*	2.67 ± 0.57 ^{Ca}	3.25 ± 0.85 ^{Ea}	3.68 ± 0.82 ^{DEa}	6.00 ± 0.70 ^{BCa}	6.83 ± 0.42 ^{Ga}	6.30 ± 0.35 ^{Da}
	ΔE	–	1.70 ± 0.41 ^{CDE}	2.31 ± 0.63 ^{BC}	–	3.47 ± 0.98 ^{ABC}	2.40 ± 0.20 ^{BCD}
ACE + GI	L^*	40.10 ± 1.54 ^{CDEa}	41.43 ± 0.45 ^{FGa}	41.70 ± 0.26 ^{CDEa}	44.40 ± 1.60 ^{Aa}	40.35 ± 1.08 ^{Ea}	41.50 ± 0.30 ^{EFa}
	a^*	−4.13 ± 0.53 ^{Ca}	−3.67 ± 0.31 ^{Fa}	−3.67 ± 0.50 ^{EFa}	0.97 ± 0.15 ^{Fc}	0.43 ± 0.10 ^{Hb}	0.10 ± 0.00 ^{Ga}
	b^*	3.23 ± 0.88 ^{Ca}	4.47 ± 0.86 ^{Fa}	3.00 ± 0.53 ^{CDa}	8.67 ± 0.67 ^{Ec}	6.55 ± 0.48 ^{Ga}	7.57 ± 0.25 ^{EFb}
	ΔE	–	2.04 ± 0.32 ^{E}	1.80 ± 0.14 ^{ABC}	–	4.60 ± 1.17 ^{BCD}	3.17 ± 0.33 ^{DE}
CT + XI	L^*	39.54 ± 1.25 ^{BCDa}	38.73 ± 0.80 ^{Ba}	39.23 ± 0.81 ^{Aa}	39.75 ± 2.39 ^{Ab}	36.85 ± 0.64 ^{Bb}	34.10 ± 1.12 ^{Aa}
	a^*	−6.55 ± 0.80 ^{Aa}	−6.40 ± 0.36 ^{ABa}	−4.57 ± 0.91 ^{CDb}	−5.51 ± 1.02 ^{Aa}	−4.35 ± 1.2 ^{Ca}	−3.53 ± 0.17 ^{Aa}
	b^*	1.45 ± 0.31 ^{Ab}	1.00 ± 0.24 ^{Aa}	2.57 ± 0.06 ^{BCc}	3.77 ± 0.51 ^{Aa}	4.40 ± 0.14 ^{CDa}	4.23 ± 0.39 ^{ABa}
	ΔE	–	1.20 ± 0.31 ^{BC}	2.43 ± 0.73 ^{C}	–	3.33 ± 0.11 ^{AB}	6.02 ± 1.08 ^{F}
EO + XI	L^*	40.44 ± 0.74 ^{DEa}	39.83 ± 0.79 ^{CDa}	40.58 ± 0.43 ^{ABCa}	40.48 ± 2.30 ^{Aa}	40.5 ± 0.76 ^{Ea}	39.83 ± 2.75 ^{DEa}
	a^*	−7.30 ± 0.30 ^{Aa}	−6.80 ± 0.32 ^{Ab}	−6.08 ± 0.22 ^{Ac}	−2.10 ± 0.29 ^{BCDb}	−6.65 ± 0.33 ^{Aa}	−2.35 ± 0.35 ^{Bb}
	b^*	1.90 ± 0.33 ^{ABa}	1.35 ± 0.21 ^{ABa}	2.05 ± 0.13 ^{ABa}	5.36 ± 0.77 ^{BCb}	1.70 ± 0.40 ^{Aa}	5.63 ± 0.56 ^{CDb}
	ΔE	–	1.28 ± 0.11 ^{BCD}	1.30 ± 0.22 ^{A}	–	5.89 ± 0.20 ^{DE}	3.00 ± 0.04 ^{CDE}
AE + XI	L^*	41.85 ± 1.13 ^{Fa}	41.55 ± 0.24 ^{Ga}	42.98 ± 2.33 ^{Ea}	42.10 ± 2.11 ^{Aa}	39.05 ± 1.77 ^{CDEa}	43.93 ± 0.38 ^{Ga}
	a^*	−6.70 ± 0.47 ^{Aa}	−5.95 ± 0.29 ^{BCb}	−3.55 ± 0.35 ^{Fc}	−1.30 ± 0.17 ^{DEb}	−5.73 ± 0.51 ^{Ba}	−1.28 ± 0.05 ^{CDEb}
	b^*	2.97 ± 0.50 ^{Ca}	2.90 ± 0.27 ^{DEa}	5.20 ± 0.14 ^{Fb}	6.18 ± 1.00 ^{CDb}	2.48 ± 0.22 ^{Ba}	6.80 ± 1.12 ^{EFb}
	ΔE	–	0.88 ± 0.25 ^{AB}	4.73 ± 1.38 ^{D}	–	6.70 ± 0.64 ^{E}	2.12 ± 0.61 ^{B}
ACE + XI	L^*	41.20 ± 0.80 ^{EFa}	39.88 ± 0.15 ^{CDa}	41.30 ± 0.89 ^{BCDa}	40.13 ± 1.78 ^{Aa}	38.15 ± 1.97 ^{BCDa}	40.40 ± 1.15 ^{EFa}
	a^*	−6.65 ± 0.40 ^{Aa}	−6.38 ± 0.29 ^{ABa}	−4.28 ± 0.21 ^{DEb}	−2.30 ± 0.10 ^{BCb}	−5.63 ± 0.75 ^{Ba}	−2.43 ± 0.71 ^{Bb}
	b^*	2.48 ± 0.50 ^{BCa}	1.90 ± 0.26 ^{ABCa}	2.60 ± 0.33 ^{BCa}	5.41 ± 0.85 ^{BCb}	2.25 ± 0.52 ^{ABa}	5.50 ± 0.46 ^{CDb}
	ΔE	–	1.51 ± 0.10 ^{CDE}	2.52 ± 0.22B ^{C}	–	5.31 ± 0.60 ^{D}	1.18 ± 0.33 ^{A}

[1]Numbers are means ± standard deviations from triplicate samples.Within each row for each tested type, means with the same lowercase letter are not significantly different (P > 0.05). Within each column, means with the same uppercase letter are not significantly different (P > 0.05).

γ-irradiation. Regarding interior lightness, such effectiveness was observed when EO was added to the sausage formulation although its encapsulation form enhanced lightness more effectively. Similarly, interior lightness increment under the effect of γ-irradiation was more pronounced than X-irradiation. But AE + XI as well as ACE + GI showed better effect on lightness improvement compared to other formulations. Among non-irradiated samples, AE and the control had the highest and the lowest values of L^*, respectively.

As can be seen in Table 2, all treatments applied in this study decreased the color coordinate of a^* on the surface significantly. Apparently, the change in a^* value resulting from X-ray was higher than γ-ray treatment as compared to the control. The introduction of EOs into the product did not affect the exterior and interior redness but its encapsulation with polymers created notable increments. By comparing the redness of EO, AE, and ACE with X- and γ-ray, the EOs and its encapsulation further decreased the exterior and interior redness and even more decrease was observed among the a^* values of samples treated with X-ray than γ-ray. By considering the common phenomenon of decreasing redness during storage time, the redness was noticed to be stable for surface of non-irradiated groups and inside of X-ray treated samples.

In all treated and untreated samples, storage time had no strong impact on the interior and exterior b^* parameter. The introduction of EOs in encapsulation created more notable increment of the surface and inside yellowness. X- or γ-irradiation did not change significantly the exterior or interior yellowness. But the combination of the EOs free or encapsulated with X- or γ-ray showed a significant difference on yellowness except for exterior yellowness after drying. Regarding interior yellowness, a wider range of b^* values was observed. Interior yellowness increment under the effect of γ-irradiation was more than X-irradiation. EO + GI showed similar yellowness while ACE + GI showed the greatest b* values compared to control.

The incorporation of EOs and their encapsulated form retained the exterior color difference of samples unchanged during 2 months of storage. Regarding the interior of the products, the total color difference within the samples of EO and AE was notably reduced during 1 month but the ACE prevented color changes, although there was no difference among all formulated samples with free and encapsulated EO, and control at 8^{th} week. X- and γ-ray did not affect the exterior total color changes but the interior total color change was adversely affected at the end of storage. Apparently, the changes made by X-ray were more pronounced than γ-ray as compared to the control. This increase was observed mostly on interior total color change at 4^{th} week of EO, AE, ACE with X- and γ-ray. The ΔE values of EO free or encapsulated with X-ray were higher than those of samples treated with γ-irradiation. The values of exterior ΔE decreased when treated with X- and γ-ray during storage and the reduction was much more obvious for interior color change. X-ray affected more the color than γ-ray and irradiation in presence of EOs affected more the color than only irradiated samples. AE and ACE with X-ray showed the highest surface total color change and the lowest interior total color change at the end of storage.

Generally, the color of irradiated meat products can vary depending on the radiation source, radiation dose, animal species of raw meat, muscle type, packaging type, and myoglobin concentration (Ham et al., 2017). Nitrosomyoglobin is usually the main pigment that causes redness in meat products containing nitrite (Ham et al., 2017). The effect of irradiation on the color change of fermented sausages has not been determined. It is thought that the reduction in redness during storage may be due to the destruction of both nitrosoheme and nitrosomyoglobin by irradiation (Kim et al., 2012). On the other hand, phenolic compounds can interrupt the oxidation reaction of irradiated meat by providing hydrogen atoms or quenching free radicals, thereby avoiding the color and texture changes caused by irradiation (Ahn et al., 2013). Ben Fadhel et al. (2016) observed that 1 kGy γ-irradiation caused a reduction in a^* value which was indicative of a significant greenness of fresh pork meat. But the green pigments were not stable when the irradiation doses increased to 1.5 and 3 kGy, while the red color related to the formation of heme pigment-CO ligand was more stable. These results are consistent with our results related to the effect of 1.5 kGy γ-irradiation on redness of DFS. According to Kim et al. (2012), redness of beef sausage patties was not affected by γ-irradiation lower than 1 kGy but decreased during storage when irradiation increased to 2 and 4 kGy. Song et al. (2017) observed similar a^* values after γ- and X-ray irradiation for low-salt sausages although the values significantly lowered at the end of refrigerated storage.

Samples irradiated with X-ray exhibited a reduction in redness accompanied by more greenness compared to the control and γ-irradiated samples. Color changes may be due to the intrinsic sensitivity of myoglobin molecules to the energy caused by irradiation (Ben Fadhel et al., 2016). Myoglobin can be bound to oxygen to form bright red oxygenated myoglobin. After irradiation, free binding sites can react with free radicals such as hydroxyl (-OH) and sulphuryl (-SH) radicals to form metmyoglobin (brown) and thiomyoglobin (green), respectively (Ouattara, Giroux, Smoragiewicz, Saucier, & Lacroix, 2002). Besides, meat color stability depends on the residual enzymatic activity in meat which controls myoglobin oxygenation, oxidation and reduction (Rodrigues et al., 2020). Hydroxyl radicals produced by ionizing radiation are considered to be a factor in accelerating lipid oxidation, which adversely affects the color, flavor, texture and nutritional value of meat (Ham et al., 2017; Park et al., 2010). Therefore, the lipid oxidation reinforces meat discoloration (Faustman, Sun, Mancini, & Suman, 2010). Ham et al. (2017) found that X-ray irradiation resulted in significantly higher TBARS values and lower a^* values than γ-ray for all doses applied on pork sausages than γ-ray. The a^* value of beef was observed initially lower in X-ray irradiated samples, but the difference was disappeared with extended storage (Kim et al., 2018).

However, further research should be conducted clearly to determine the effect of different irradiation sources and dose levels on nitrosyl hemochrome and endogenous enzyme stability and free radical generation in dry fermented and irradiated meat products.

3.7. Texture evaluation

The textural characteristics including maximum stress and Young's modulus of the sausages processed with EOs free or encapsulated and combined treatments of EO and irradiation are presented in Table 3. Maximum force during the compression showed no difference in treatments of EO, AE and ACE. Young's modulus was not also affected by the storage time like maximum stress. After drying, ACE samples were more elastic than other samples but all samples adopted the similar values of elasticity after 8 weeks indicating that the antimicrobial formula treatments had no effects on hardness compared to control. The γ-irradiation had no effect on maximum stress and Young's modulus during storage. It should be noted there were some fluctuations in elasticity for the irradiated samples of ACE and the control.

X-ray irradiation showed an increase of Young's modulus during storage. This effect was also observed for breaking stress. But it decreased for the X-ray with AE and ACE. It is observed in this study that the presence of encapsulating polymers contributed to the softness of the texture. Interestingly, all samples irradiated with X-ray adopted the highest hardness in the middle of their storage (4^{th} week) and after 1 month, they followed a decreasing trend which is indicative of vital role of aging for processed sausages. EO, AE and ACE samples with γ-ray groups had no significant difference on maximum stress and modulus compared to non-irradiated counterpart groups at 8^{th} week of storage. AE + XI and ACE + XI showed higher modulus values than the counterpart groups after drying. Non-irradiated groups all showed stable maximum stress and Young's modulus during storage. Groups treated with γ-ray and X-ray also showed stable maximum stress during storage.

The hardness of X-ray treated samples were significantly higher than control and γ-ray treated samples initially, but the differences reduced during storage. In a study conducted by Houben and van't Hooft (2005),

Table 3
Max stress (MPa) and Young's modulus (MPa) of sausages measured during storage at room temperature[1].

	Max stress (MPa)			Young's modulus (MPa)		
Samples	After drying	4weeks	8weeks	After drying	4weeks	8weeks
CT	0.26 ± 0.03 ^{CDEa}	0.21 ± 0.05 ^{Aa}	0.23 ± 0.01 ^{Ba}	7.04 ± 0.91 ^{DEa}	5.18 ± 0.94 ^{ABa}	5.57 ± 1.07 ^{ABa}
EO	0.21 ± 0.03 ^{ABCDa}	0.27 ± 0.01 ^{ABCa}	0.28 ± 0.03 ^{BCDa}	5.55 ± 0.73 ^{BCDa}	5.4 ± 0.19 ^{ABa}	5.57 ± 0.10 ^{ABa}
AE	0.24 ± 0.01 ^{BCDEa}	0.29 ± 0.04 ^{BCa}	0.31 ± 0.06 ^{DEa}	6.16 ± 0.80 ^{BCDa}	7.20 ± 1.31 ^{BCa}	5.45 ± 0.04 ^{ABa}
ACE	0.20 ± 0.01 ^{ABa}	0.28 ± 0.07 ^{ABCa}	0.31 ± 0.07 ^{DEa}	4.47 ± 0.65 ^{ABa}	7.98 ± 1.24 ^{CDb}	5.30 ± 1.15 ^{ABa}
CT + GI	0.27 ± 0.04 ^{CDEa}	0.24 ± 0.01 ^{ABa}	0.18 ± 0.04 ^{Aa}	5.97 ± 0.36 ^{BCDc}	3.56 ± 0.34 ^{Aa}	4.48 ± 0.14 ^{Ab}
EO + GI	0.21 ± 0.00 ^{ABCa}	0.30 ± 0.07 ^{BCa}	0.31 ± 0.04 ^{CDEa}	4.46 ± 0.27 ^{ABa}	7.09 ± 0.93 ^{BCa}	5.76 ± 1.15 ^{ABa}
AE + GI	0.27 ± 0.06 ^{DEa}	0.25 ± 0.03 ^{ABCa}	0.30 ± 0.02 ^{CDEa}	6.70 ± 0.64 ^{CDEa}	5.97 ± 0.31 ^{BCa}	5.43 ± 0.26 ^{ABa}
ACE + GI	0.15 ± 0.01 ^{Aa}	0.31 ± 0.06 ^{Ca}	0.28 ± 0.06 ^{BCDa}	3.31 ± 0.51 ^{Aa}	5.80 ± 0.82 ^{BCb}	5.63 ± 0.52 ^{ABb}
CT + XI	0.30 ± 0.01 ^{Ea}	0.41 ± 0.05 ^{Db}	0.23 ± 0.03 ^{Ba}	10.43 ± 1.18 ^{Fb}	14.85 ± 0.21 ^{Ec}	8.18 ± 1.55 ^{Ca}
EO + XI	0.37 ± 0.05 ^{Fa}	0.50 ± 0.02 ^{Eb}	0.46 ± 0.02 ^{Fb}	10.05 ± 1.85 ^{Fa}	13.35 ± 3.06 ^{Ea}	6.68 ± 0.85 ^{BCa}
AE + XI	0.25 ± 0.03 ^{BCDEa}	0.45 ± 0.02 ^{DEc}	0.36 ± 0.02 ^{Eb}	8.22 ± 1.38 ^{Eb}	12.82 ± 2.42 ^{Ec}	4.46 ± 0.32 ^{Aa}
ACE + XI	0.21 ± 0.04 ^{ABCa}	0.29 ± 0.01 ^{BCc}	0.25 ± 0.01 ^{BCb}	4.97 ± 0.60 ^{ABCa}	9.45 ± 0.61 ^{Db}	5.97 ± 0.68 ^{ABa}

[1]Numbers are means ± standard deviations from triplicate samples. Within each row for each tested type, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column, means with the same uppercase letter are not significantly different ($P > 0.05$).

the role of storage time in reducing of maximum stress and Young's modulus values was confirmed. Irradiation of beef can induce oxidative conditions and promote protein oxidation, leading to myofibril protein denaturation and aggregation, and loss of proteolytic enzyme activity (Kim et al., 2018). It has been also observed that calpain-1 (a proteolytic enzyme found in meat that is the major enzyme for the degradation of myofibrillar proteins and contributes to the development of meat softness) has a low degree of autolysis, therefore the enzyme activity is less extensive in samples irradiated by X-rays (Kim et al., 2018). Also, degradation fragments of calpain-14-like were found in prok muscles at 3 kGy γ-irradiation and a complete inactivation of calpains may occur at higher dose of γ-irradiation (≥5 kGy) (Zhang et al., 2020). In present test, X-ray may affect more calpain autolysis activity than γ-ray that resulted in higher hardness. It may also relate to higher lipid oxidation induced by X-ray than γ-ray. Compared to the primary products of lipid oxidation (such as hydroperoxides), amino acids are more susceptible to be damaged by secondary products of lipid oxidation,which can interact with amino acid residues of proteins therefore affect protein structure and function (Zhang, Xiao, & Ahn, 2013). As known, oxidative stress caused by irradiation is very obvious in meat products. However, few studies have been conducted about the effect of irradiation on the oxidation of muscle proteins from the perspective of proteomics and its potential contribution to the development of softness during further storage (Zhang et al., 2020). Also, the effect of low-to-medium-dose irradiation on the quality of dry fermented beef products is not yet clear, because most studies have been conducted on ground beef, which responds differently to irradiation in terms of lipid oxidation and color changes.

4. Conclusion

Encapsulation of alginate and alginate-CNC did not show any considerable effect on microbial quality of dry fermented sausages. Synergistic antimicrobial effects were observed for EOs or EOs encapsulated combined with 1.5 kGy γ-irradiation against *E. coli* O157:H7 cocktail and LAB, and when combined with 1.5 kGy X-ray against *E. coli* O157:H7 cocktail. Combined treatments of EOs with ionizing radiation showed strong inhibition on *L. monocytogenes*, Y/M and TMF. Encapsulation contributed to the protection of sausage color during storage. The combined treatments with γ-irradiation did not affect the physicochemical quality of sausages. When EO formulas were combined with X-ray, a reduction of redness was observed and higher hardness was noticed at initial time of storage but aging process diminished the observed differences. This research provides novel methods of combining free or encapsulated EOs and high dose of γ-ray or low dose of X-ray ionizing radiation for preserving the ready-to-eat meat by food industry and can be used for safety of other kinds of food products such as ground meat and burgers. However, there are still some challenges related to use of EOs in different food products due to their aromatic profiles, and possible alterations in the sensory qualities of products. Therefore, future studies are required to determine the effect of EOs on the sensory qualities of food products and the evaluation of consumer acceptability.

CRediT authorship contribution statement

Jiali Ji: Investigation, organizing of the database, performing the statistical analysis, Writing – original draft, preparation. **Zahra Allahdad:** Writing – review & editing. **Elham Sarmast:** Manuscript revision, Review. **Stephane Salmieri:** Methodology, Resources, Manuscript revision, Review. **Monique Lacroix:** Conceptualization, Methodology, Supervision, Manuscript revision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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