SHELF-LIFE DETERMINATION OF DRY FERMENTED POULTRY MEAT SAUSAGE USING ARRHENIUS MODEL

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Abstract

The effect of commercial starter cultures and essential oils (EOs) on the shelf-life of dry fermented poultry meat sausage was investigated. A total of 30 sausages were inoculated with two different mixed starter cultures and two different EOs: 10^8 UFC.g^{-1} *L. sakei* + *S. carnosus* (starter A), 10^8 UFC.g^{-1} *L. sakei* + *S. carnosus* + *S. xylosus* (starter B), 0.25% (v/v) of oregano EO (EO 1) and 0.25% (v/v) of thyme EO (EO 2), and stored for 28 days at three different temperatures 15, 25 and 35 °C. Shelf-life evaluation was determined by using Accelerated Shelf-Life Testing (ASLT) method of Arrhenius model. Fecal coliforms counts were used as a parameter in this research. The results indicated that storage time affected (P < 0.05) the evolution of fecal coliforms counts whereas no significant differences were observed (P > 0.05) among the samples inoculated with starter cultures and added with EOs. Based on the microbial counts, the prior addition of mixed starter cultures A or B to dry fermented sausages, incubated at 15 °C, has improved their shelf life for about 12 days against 7 days for sausages added with oregano or thyme essential oils, and this compared with a shelf life of 20 days noted on control sample.

Key Words: Dry fermented sausage; Shelf life; Essential oils; Starter cultures; Fecal coliforms.

1. Introduction

Processed meat products are very sensitive to microbial recontaminations. Consumers demand a reduced use of chemical preservatives and also high hygienic and organoleptic quality of foods. Hence, biopreservation has received considerable attention as a means of naturally controlling the shelf life and safety of meat products. Currently, lactic acid bacteria (LAB) and antimicrobial compounds synthesized by LAB are considered to be natural preservatives or biopreservatives (Casaburi et al., 2008; Tabanelli et al., 2012; El Adab et al., 2015; Ammor and Mayo, 2017). In fact, during ripening of fermented meat products, LAB ferment glucose to lactic acid, which is responsible for the pH decrease. This acidification contributes to the formation of the specific acidic taste of the final product (Parente et al., 2001; Drosinos et al., 2007; Leroy et

al., 2006, Casaburi et al., 2008). Besides, lactic acid bacteria inhibit the growth of pathogenic microorganisms by the production of antimicrobial compounds other than lactic acid such as bacteriocins, acetic acid, ethanol, acetoin and hydrogen peroxide. Thus, LAB improve the safety, stability and shelf life of meat products (Gao et al., 2014; El Adab et al., 2015).

Moreover, the use of volatile oily extracts, which are called biopreservatives or green chemicals, has increasingly gained the interest of researchers and food processors as potential alternatives to chemical preservatives (Bensid et al., 2014; Jouki et al., 2014). In fact, these natural products have been shown to possess antibacterial and antifungal activities (Ouattara et al., 1997) against several microorganisms associated with meat, including gram-negative and gram-positive bacteria (Karabagias et al., 2011).

The study of the stability of food products is often carried out using the Accelerated Shelf-Life Testing (ASLT) method which is used for estimating the shelf life of perishable food (Labuza, 1984). The goal of this research is to study the effect of commercial starter cultures and essential oils (EOs) on the shelf-life of dry fermented poultry meat sausage.

2.   Material and methods

2.1.   Preparation of dry fermented sausages

The sausage formulation included 11.250 kg of poultry meat (75%), 3.750 kg of poultry fat (25%), 600 g of salt, 30 g of black pepper, 30 g of paprika, 150 g glucose and 1.5 g of potassium nitrate. After chopping and mixing the ingredients, the mixture was divided into five batches (3 kg for each batch): batch 1, inoculated with a commercial starter culture starter A (20 g/200 kg): *L. sakei + S. carnosus + S. xylosus* (TEXEL SA-201, DANISCO, Paris, France); batch 2, inoculated with a commercial starter culture starter B (25 g/100 kg): *L. sakei + S. carnosus* (BFL-F06, CHR HANSEN, Nienburg, Germany); batch 3, contained 0.25% (v/v) oregano EO (*Coridothymus capitatus*, Pharmacy Makni, Manouba, Tunisia); batch 4, contained 0.25% (v/v) thyme EO (*Thymus vulgaris*, Pharmacy Makni) and batch 5, control without inoculation. Starter A and starter B were added to sausages according to manufacturer's recommendations. The mixture of each batch was stuffed into artificial casings and then placed in a fermentation chamber (BCR, CF 1 B, Antony, France). The sausages were fermented for 5 days at 24 °C and 80% relative humidity (RH). After 5 days of processing, the temperature was decreased to 14 °C for 23 days and the RH value was 80%. Then, the sausages were stored for 28 days at three different temperatures 15, 25 and 35 °C. For sampling, three sausages of each batch at 0 day (mix before storage) and after 7, 14, 21 and 28 days of storage were taken for microbiological analyses, and each analysis was carried out in triplicate.

2.2.   Microbiological Analysis

Sausage samples (10 g) of each batch were homogenized with 90 mL of sterile peptone water (Biolife, Milan, Italy) and decimal dilutions were prepared. Fecal coliforms were determined on desoxycholate (0.1%) lactose agar (Biokar) at 44 °C for 24 h.

2.3.   Shelf-Life Determination

Chemical kinetics can be applied in food science for the prediction of the change in quality of a food as a function of time and environmental conditions (Labuza, 1984). The shelf-life of the sausage samples was determined. The technique consisted of an Accelerated Shelf-Life Testing (ASLT) of the sausage samples. The end of the shelf-life of foods has often been related to the microbiological counts and/or values of physicochemical parameters in different products categories (Calligaris et al., 2007).

Therefore, in this study, fecal coliforms count was chosen as the alteration factor determining the shelf life of the sausage samples. For this purpose, sausage samples were stored for 28 days at 3 different storage temperatures (15, 25, and 35 °C). The Arrhenius model was used to describe the deterioration kinetics of dry fermented sausages:

$$d[A]/dt = K [A]^n$$

A: represents the number of fecal coliforms (CFU/g);

t: storage time (days);

K: specific rate of reaction (day^{-1});

n: is the power factor called reaction order (first order or zero order for foods).

Moreover, the Arrhenius equation can be used to express the rate constant as a function of temperature:

$$Ln\ K = (-E_a/R) \times (1/T) + Ln\ K_0$$

Where:

E_a: Arrhenius activation energy ($J.\ mol^{-1}$);

R: perfect gas constant ($R = 8.314\ J.\ mol^{-1}\ K^{-1}$);

T: temperature (°K).

The stages to determine products shelf-life based on ASLT Arrhenius method including: (a) Plotting the parameter value as temperature function in zero order and first order; (b) Plot of the result of both orders will produce slope value (K_0), intercept, correlation coefficient (R^2); (c) Plot to Arrhenius equation (1/T, ln K); (d) K value, intercept and correlation coefficient are resulted. K value is the gradient of linear regression which is resulted from three storage conditions (Córdova et al., 2011).

2.4.   Statistical Analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) procedure of SPSS 17.0 (SPSS, Inc., Chicago, IL). Duncan's multiple range test was used to determine any significant difference between mean values and evaluations were based on a significance level of $P < 0.05$.

3.   Results and discussion

Our approach to calculate the shelf-life of the investigated sausage samples is based on the Arrhenius model as reported by Labuza (1984). Fecal coliforms count was chosen as the alteration factor determining the shelf life of the sausage samples.

$$d[A]/dt = K [A]^n$$

A: Number of fecal coliforms (CFU/g);

t: Storage time (days);

n: Degree of the equation which is of first order or zero order for foods;

K: Specific rate of the bacterial deterioration reaction ($days^{-1}$).

The integration of this equation gives two types of functions according to the value of n:

- $[A] = [A_0] - Kt$; for $n = 0$;
- $Ln [A] = -Kt + Ln [A_0]$; for $n = 1$.

With:

A_0: Initial number of fecal coliforms (CFU/g) measured at the initial time (t = 0) of storage.

The linear representations of the numbers of fecal coliforms, expressed in CFU/g and Ln (CFU/g), as a function of the storage time (days) of control sausages and sausages inoculated with mixture starter cultures and added with essential oils make it possible to determine whether the reaction order of the bacterial deterioration of these products is 0 or 1 (Fig. 1). The results indicated that storage time affected ($P < 0.05$) the evolution of fecal coliforms counts whereas no significant differences were observed ($P > 0.05$) among the samples inoculated with starter cultures and added with EOs. Our results are in agreement with many other studies reporting that storage time affected ($P < 0.05$) the evolution of all microbial groups (Cachaldora et al., 2013; Golestani et al., 2017; Marcelo et al., 2017).
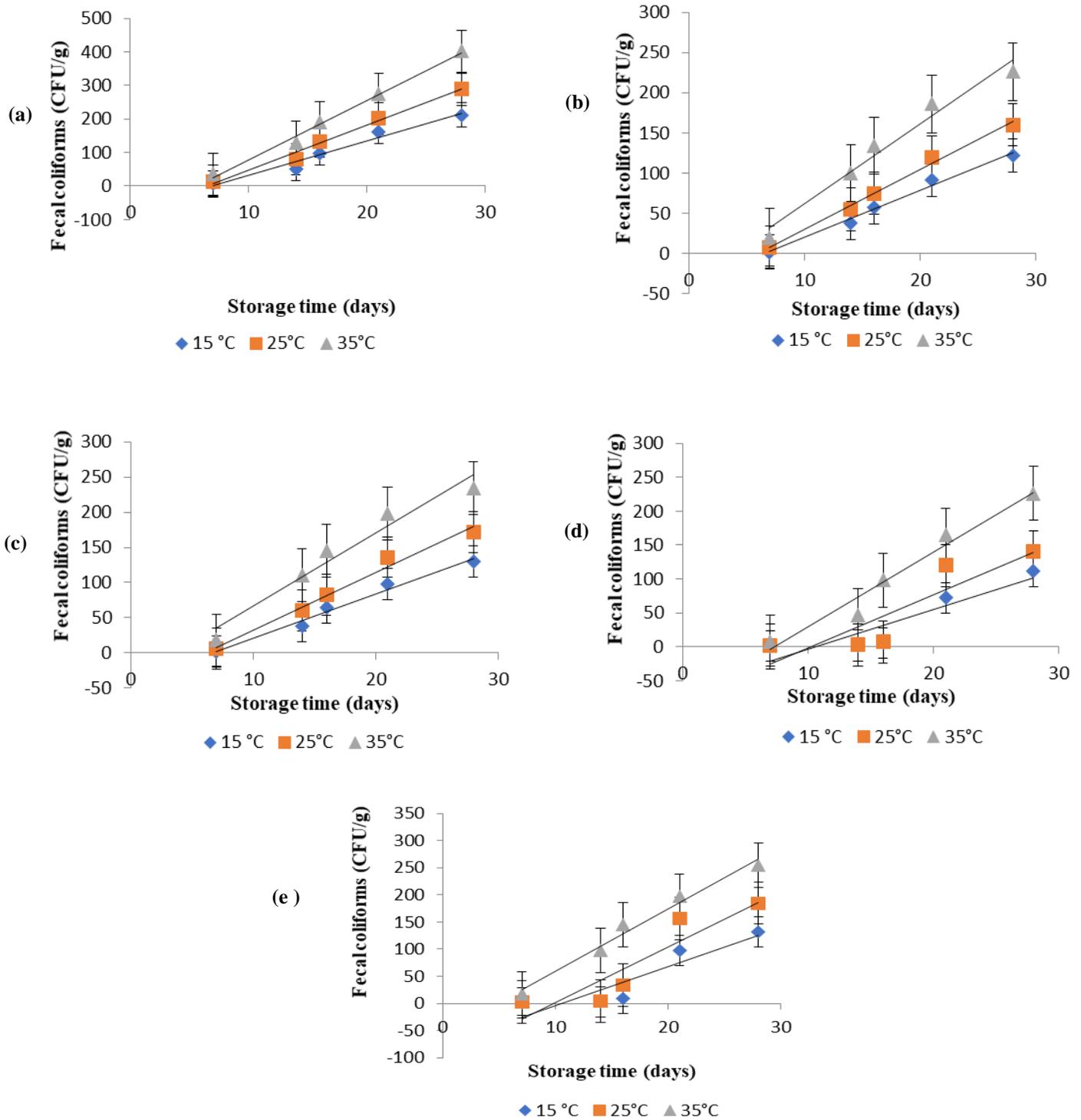
Fig 1. Evolution of fecal coliforms during the storage of dry fermented sausages: a (control sausage), b (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*), c (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), d (sausage added with oregano EO), e (sausage added with thyme EO)

The equations of the corresponding simple linear regressions have also been calculated (Table 1).

Table 1- Equations of the simple linear regressions established between the numbers of fecal coliforms (CFU/g and Ln CFU/g) as a function of the storage time (days) of dry fermented sausages

Temperature (°C)	Control sausages				Sausages inoculated with S.c + L.s				Sausages inoculated with S.c + S.x + L.s				Sausages added with oregano essential oil				Sausages added with thyme essential oil			
	Zero order		First order		Zero order		First order		Zero order		First order		Zero order		First order		Zero order		First order	
	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2	(K)	R^2
15	10.33	0.97	0.16	0.84	5.89	0.99	0.18	0.77	6.31	0.98	0.19	0.77	5.87	0.84	0.25	0.89	7.16	0.83	0.26	0.91
25	13.44	0.99	0.14	0.86	7.44	0.99	0.14	0.83	8.19	0.98	0.15	0.79	7.82	0.78	0.24	0.84	10.19	0.74	0.24	0.84
35	17.71	0.99	0.11	0.90	9.98	0.97	0.11	0.81	10.4	0.96	0.11	0.77	11.07	0.97	0.16	0.86	11.44	0.96	0.12	0.82

S.c + S.x + L.s: Sausage inoculated with mixed starter culture B (*S. carnosus* + *S. xylosus* + *L. sakei*); S.c + L.s: Sausage inoculated with mixed starter culture A (*S. carnosus* + *L. sakei*).

Independently of the incubation temperature applied, the analysis of the results obtained shows clearly that the bacterial deterioration equation of the products studied is of first order (R^2 close to 1). Accordingly, this order will be considered for the remainder of the study. Thus, the equation of the bacterial deterioration of dried sausages in first order is written:

$$Ln [A] = -Kt + Ln [A_0]$$

Moreover, the Arrhenius equation can be used to express the rate constant (K) of the bacterial deterioration reaction as a function of the temperature applied:

$$Ln K = (-Ea/R) \times (1/T) + Ln K_0$$

Thus, for each temperature used, the rate constant (K) was determined experimentally (Table 2). The linear representations, established between the Ln of the rate constants of the bacterial deterioration reaction of the sausages studied as a function of the inverse of temperature (Fig. 2) allowed us to deduce the values of (E_a/R) (slope of the line) and the values of K_0 (abscissa at the origin).

On the other hand, the equation of the rate constant can be written:

$$K = K_0 \times e^{(-Ea/RT)}$$

The equation of the bacterial deterioration of dried sausages in first order is written:

$$Ln [A] = -Kt + Ln [A_0];$$ we deduce that:
$$[A] = [A_0] \times e^{(-Kt)};$$

According to this equation, the shelf life is expressed according to the following formula:

$$t (days) = - (Ln(A) - Ln(A_0))/K_0 e^{(-Ea/RT)})$$

With:
t: Estimated shelf life of dry fermented sausages (days);
$[A_0]$: Number of fecal coliforms on day 0 of conservation;
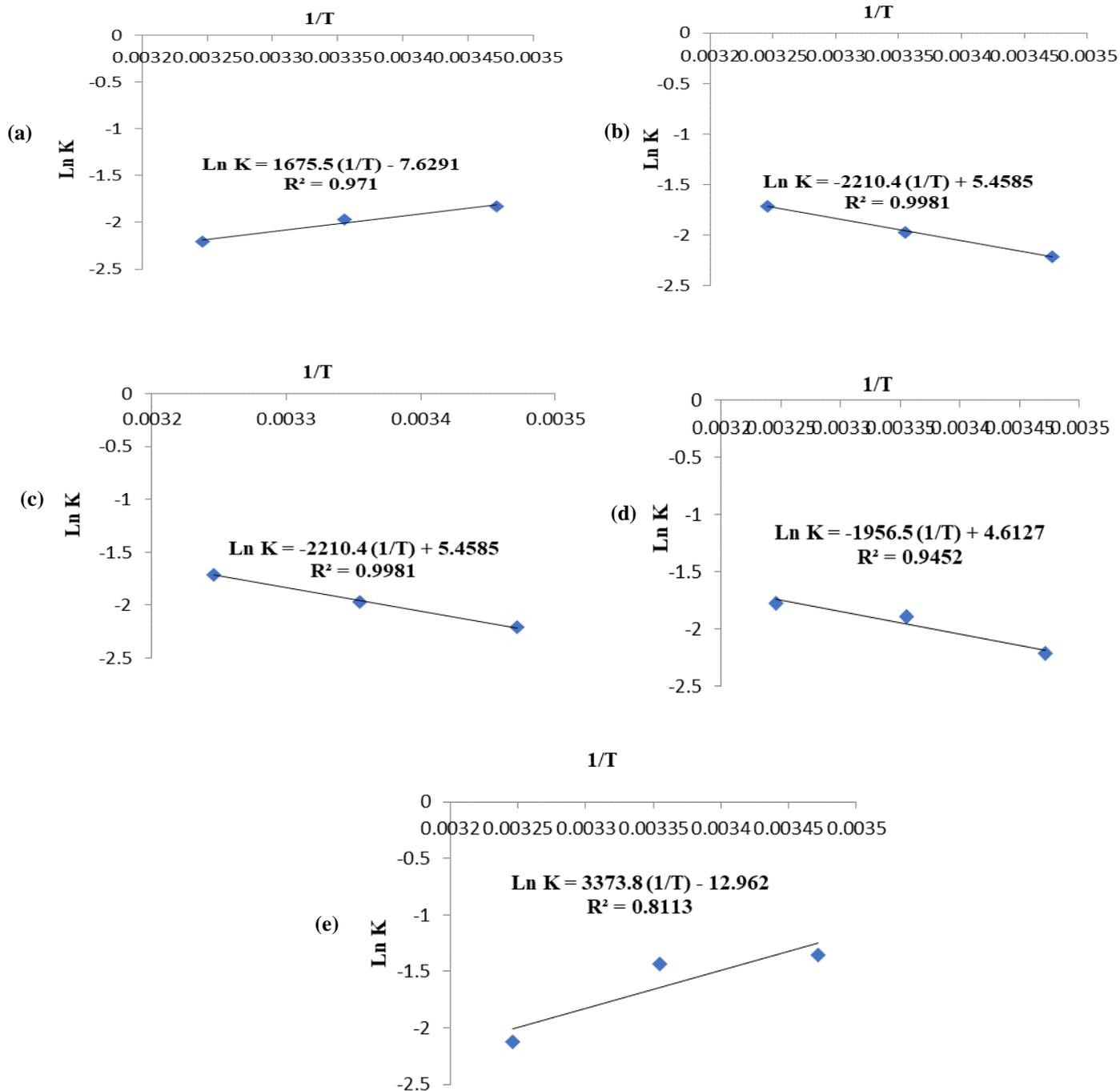$[A]$: European standard limit of fecal coliforms in dry sausages.

Fig 2. Plot of the rate constant as a function of reciprocal absolute temperature (1/T), a (control sausage), b (sausage inoculated with mixed starter culture *S. carnosus* and *L. sakei*), c (sausage inoculated with mixed starter culture *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Lactobacillus sakei*), d (sausage added with oregano EO), e (sausage added with thyme EO).

Table 2- Rate Constants (K) relative to the evolution of fecal coliforms (Ln CFU/g) during storage of dry fermented sausages

Temperature (°K)	1/T (°K^{-1})	control sausages	Sausages inoculated with S.c + L.s	Sausages inoculated with S.c + S.x + L.s	Sausages added with oregano essential oil	Sausages added with thyme essential oil
		Ln K	Ln K	Ln K	Ln K	Ln K
288	0.003472	-1.83	-1.71	-1.66	-1.39	-1.35
298	0.003355	-1.97	-1.97	-1.89	-1.43	-1.43
308	0.003246	-2.21	-2.21	-2.21	-1.83	-2.12

S.c + S.x + L.s: Sausage inoculated with mixed starter culture B (*S. carnosus + S. xylosus + L. sakei*); S.c + L.s: Sausage inoculated with mixed starter culture A (*S. carnosus + L. sakei*).

The shelf life values of sausages analyzed are summarized in Table 3. Our results showed that, regardless of the incubation temperature applied, the shelf life values obtained for the inoculated dry sausages remains higher than those noted on control sausages or sausages added with oregano and thyme essential oils. Thus, the prior addition of mixed starter cultures A or B to dry fermented sausages, incubated at 15°C, has improved ($p < 0.05$) their shelf life for about 12 days against 7 days for sausages added with oregano or thyme essential oils, and this compared with a shelf life of 20 days noted on control sample. This is related to the beneficial effect exerted by the essential oils or the mixed starter cultures on the decontamination of these products. Our results are in agreement with many other studies reporting that the addition of starter cultures and essential oils (EOs) to dry fermented sausages improved their shelf life (Balzan et al., 2017; Marcelo et al., 2017). In fact, the acidifying activity of lactic acid bacteria plays an important role in the stability of meat products, following the inhibition of the growth of spoilage and pathogenic microorganisms. Therefore, lactic acid acts as a bio-preservative of all fermented products. In addition, the development of color and ripening are favored in acidic medium (Bronomo et al., 2008). Aymerich et al. (2008) and Albano et al. (2009) reported that lactic acid bacteria are known and used for their antagonistic effects which they develop. These properties are mainly due to excreted metabolites, such as acetic acid, acetoin, carbon dioxide, peroxide hydrogen, diacetyl and bacteriocins. The bacteriocins produced by LAB have an activity directed against gram-positive bacteria responsible for major organoleptic defects such as *Clostridium perfringens* or *Listeria monocytogenes* (Messi et al., 2001; Lambert et al., 2001). For Gram-negative bacteria, their outer membranes do not allow the bacteriocins to reach the inner membrane, the seat of their activities (Ammor and Mayo, 2007).

Our results showed that the addition of thyme and oregano EOs to dry fermented sausages has also improved their shelf life. Lambert et al. (2001) showed that carvacrol and thymol are responsible for the antimicrobial and antioxidant activities of oregano and thyme essential oils. In fact, these compounds are able to disintegrate the outer membrane of gram-negative bacteria

by releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Helander et al., 1998). Braga et al. (2006) and El Adab and Hassouna (2016) reported that carvacrol and thymol have oxido-reducing properties and thus play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxides.

Table 3- Shelf life values (days) of dry fermented sausages, inoculated and added with essential oils at three different temperatures using ASLT method

Analyzed sample	Shelf life (days)		
	T= 288 °K	T= 298 °K	T= 308 °K
Control sausage	20.05 ± 0.01[a]	19.33 ± 0.03[b]	13.04 ± 0.06[c]
Sausage inoculated with (L.s + S.c)	32.53 ± 0.01[b]	31.21 ± 0.01[c]	27.12 ± 0.03[a]
Sausage inoculated with (L.s + S.c + S.x)	31.34 ± 0.11[b]	30.04 ± 0.21[b]	27.06 ± 0.03[a]
Sausage added with oregano essential oil	27.02 ± 0.05[c]	24.11 ± 0.02[a]	18.22 ± 0.23[b]
Sausage added with thyme essential oil	26.22 ± 0.13[c]	22.44 ± 0.23[a]	17.05± 0.02[b]

[a,b,c] Values sharing the same lowercase letter within a column are not significantly different by Duncan's multiple-range test (P < 0.05). S.c + S.x + L.s: Sausage inoculated with mixed starter culture B (*S. carnosus + S. xylosus + L. sakei*); S.c + L.s: Sausage inoculated with mixed starter culture A (*S. carnosus + L. sakei*).

Conclusion

The use of starter cultures and essential oils could be useful for maintaining hygienic quality of sausages by inhibition of spoilage and pathogenic microorganisms, which allows a good preservation of sausages and consequently improved their shelf life. Based on the microbial counts, the prior addition of mixed starter cultures A or B to dry fermented sausages, incubated at 15 °C, has improved their shelf life for about 12 days against 7 days for sausages added with oregano or thyme essential oils, and this compared with a shelf life of 20 days noted on control sample.

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