

## EFFECT OF ALIL ISOTHIOCYANATE AND GARLIC ESSENTIAL OIL AGAINST *ESCHERICHIA COLI* O157:H7 IN FRESH PORK SAUSAGE

Renata E. F. Macedo<sup>1\*</sup>, Fernanda K. Bortolotto<sup>1</sup>, Stephane Pini Costa Cecotti<sup>2</sup>, Hanna Lethycia Wolupeck<sup>2</sup> and Fernando B. Luciano<sup>1</sup>

<sup>1</sup>Graduate Program in Animal Science, School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, São José dos Pinhais, Brazil

<sup>2</sup>Laboratory of Agri-Food Technology, School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, São José dos Pinhais, Brazil

\* Corresponding author: renata.macedo@pucpr.br

**Abstract** – The meat products market shows a growing demand for natural preservatives. Thus, essential oils have been studied as potential natural preservatives. This study aimed to evaluate the effect of the addition of garlic essential oil and allyl isothiocyanate (AIT) in bulk sausage inoculated with *E. coli* O157:H7. Bulk pork sausages were inoculated with a pool of *E. coli* O157:H7 and different combinations of garlic oil and allyl isothiocyanate ranging from 125 ppm to 250 ppm. Sausages portions (100g) were stored at 6 °C for 20 days. At 5 day intervals, count of *E. coli* O157:H7 and instrumental color were assessed. Results were analyzed by ANOVA and means were compared by Tukey's test ( $P<0.05$ ). The addition of the combinations of essential oils was effective in inhibiting the growth of *E. coli* O157:H7, especially on treatments with the highest combination doses of essential oils ( $P<0.05$ ). Coordinates a\* and C\* values increased during the storage time in bulk sausages with essential oils. The concentrations of garlic oil and AIT used were able to inhibit the growth of *E. coli* O157:H7 and to maintain the red color of bulk pork sausage during 20 days of storage.

### I. INTRODUCTION

Fresh pork sausages are very popular in Brazil and their production represents 40% of the Brazilian meat products market. The process of making sausage is usually simple, requiring no sophisticated technology. However, due to its high water activity and the absence of heat treatment during processing, fresh pork sausages are susceptible to microbial spoilage and usually have short shelf life [1]. *Escherichia coli* stands out among the main microorganisms involved in foodborne diseases in Brazil. This species may harbor pathogenic serotypes such *E. coli* O157:H7, which has been associated with outbreaks involving meat products reported worldwide in recent years [2,3]. In the manufacture of sausage curing salts

(NO<sub>2</sub> and/or NO<sub>3</sub>) are employed to ensure the safety of the product. However, the curing salts are mainly effective in the inhibition of *Clostridium botulinum*, not showing significant inhibitory effect on *E. coli* and other pathogens or spoilage microorganisms [4]. Moreover, there is a growing demand for food with lower levels of synthetic additives [5]. Thus, the addition of natural compounds with antimicrobial activity in fresh sausage might be a strategy to increase its safety and healthiness [6]. Among these natural compounds, essential oils, extracted from plants, have been studied as potential natural preservatives in food [6,7,8]. However, due to their aromatic properties, when used as food preservatives, essential oils might influence the flavor and taste of the meat products. Thus, the use of essential oils extracted from spices traditionally used in formulating fresh sausages can minimize those adverse effects [5]. The objective of this study was to evaluate the effect of the addition of garlic essential oil and allyl isothiocyanate (AIT) in bulk sausage inoculated with *E. coli* O157:H7.

### II. MATERIALS AND METHODS

Allyl isothiocyanate (AIT) (Sigma Aldrich Chemical Co., St. Louis, USA), the main antimicrobial compound of mustard oil, and garlic oil (Zhengzhou Sigma Chemical Co., Henan, China), with 55.2% of allicin, were used in this study.

A pool of 5 strains of *E. coli* O157:H7 was grown for 5 h in Tryptone Soya Broth (Himedia, Mumbai, Índia) to achieve count of 7–8 log CFU/ mL and be used as the inoculum.

In a preliminary study, the combination of garlic oil and AIT in doses of 12.5 and 25 ppm showed synergistic effect against *E. coli* O157:H7 *in vitro*.

When used in food matrices, doses of essential oils obtained *in vitro* have to be increased in order to maintain the same antimicrobial effect [9,10]. Thus, doses of essential oils were increased tenfold to be tested in sausages patties against *E. coli* O157:H7 (Table 1).

Table 1. Combinations of essential oils added in bulk sausage inoculated with *E. coli* O157:H7.

Compound	Treatments				
	C	1	2	3	4
Garlic oil (ppm)	-	125	250	125	250
AIT (ppm)	-	125	125	250	250

C= control; AIT = allyl isothiocyanate

Five batches of bulk pork sausages were produced according to the industrial formulation presented in Table 2.

Table 2. Formulation of bulk pork sausage

Ingredients/ additives	Quantity (%)
Pork lean meat (leg)	92.4
Water	2.5
Salt (NaCl)	2.5
Sodium phosphate	1
Sodium erythorbate	0.8
Spices mixture	0.5
Monosodium glutamate	0.25
Curing salt (NaCl + NaNO <sub>2</sub> )	0.05
Total	100

Pork leg was ground in an electric grinder (Siemens, PC10, Brusque, Brazil) using a 8 mm diameter disc. Dry ingredients were added to the raw meat and mixed manually for 2 minutes. Subsequently, 1% inoculum, previously prepared, was added to the mixture by hand mixing for 2 minutes. Finally, garlic and allyl isothiocyanate essential oils were added in the concentrations described above. After manual mixing, portions of approx. 100g were placed in sterile plastic bags (Laborclin, 570671, Pinhais, Brazil) and stored at 6 °C for 20 days. At 5 day intervals portions were withdrawn and the count of *E. coli* O157:H7 and the determination of instrumental color were performed according to methodology described below.

Count of *E. coli* O157:H7: was performed on McConkey Sorbitol Agar (Himedia, M298, Mumbai, India) at 37 °C for 24h. Light pink colored colonies were identified as *E. coli* O157:H7.

Instrumental color: was determined using colorimeter (Konica Minolta CR 410, Tokyo, Japan), illuminant D<sub>65</sub>, opening diameter 50-53 mm, observation angle 2° and CIE color coordinates L\*, a\*, b\*, C\* and hue.

Statistical analysis: results were analyzed by ANOVA and means were compared by Tukey's

test ( $P<0.05$ ) using Statgraphics Centurion XVI version 16.1.11 program.

### III. RESULTS AND DISCUSSION

The addition of the combination of essential oils in sausage was effective in inhibiting the growth of *E. coli* O157:H7, especially on treatments with the highest combination doses of essential oils (Table 3).

Table 3. Count of *E. coli* O157:H7 (log CFU/ g) in bulk pork sausage with different combinations of garlic essential oil and allyl isothiocyanate (average  $\pm$  standard deviation)

Time (d)	Treatments				
	C	T1	T2	T3	T4
0	6.57 $\pm$ 0.15 <sup>a</sup>	6.57 $\pm$ 0.15 <sup>a</sup>	6.57 $\pm$ 0.15 <sup>a</sup>	6.57 $\pm$ 0.15 <sup>a</sup>	6.57 $\pm$ 0.15 <sup>a</sup>
5	5.91 $\pm$ 0.08 <sup>c</sup>	6.24 $\pm$ 0.14 <sup>b</sup>	6.69 $\pm$ 0.09 <sup>a</sup>	5.89 $\pm$ 0.07 <sup>bc</sup>	5.68 $\pm$ 0.15 <sup>d</sup>
10	6.36 $\pm$ 0.05 <sup>a</sup>	6.25 $\pm$ 0.30 <sup>a</sup>	5.73 $\pm$ 0.08 <sup>bc</sup>	5.74 $\pm$ 0.26 <sup>b</sup>	5.22 $\pm$ 0.14 <sup>c</sup>
15	5.92 $\pm$ 0.02 <sup>abc</sup>	6.27 $\pm$ 0.30 <sup>a</sup>	6.01 $\pm$ 0.21 <sup>ab</sup>	5.42 $\pm$ 0.27 <sup>bc</sup>	5.32 $\pm$ 0.36 <sup>c</sup>
20	6.27 $\pm$ 0.13 <sup>a</sup>	6.16 $\pm$ 0.04 <sup>ab</sup>	5.86 $\pm$ 0.32 <sup>ab</sup>	5.56 $\pm$ 0.45 <sup>b</sup>	4.70 $\pm$ 0.17 <sup>c</sup>
R	0.30	0.41	0.71	1.01	1.87

C= control; AIT = allyl isothiocyanate; T1 = 125 ppm garlic oil + 125 ppm AIT, T2 = 250 ppm garlic oil + 125 ppm AIT; T3 = 125 ppm garlic oil + 250 ppm AIT, T4 = 250 ppm garlic oil + 250 ppm AIT. Different letters in the same line indicate significant differences among treatments ( $P<0.05$ ). R = reduction on initial count

The counts of *E. coli* O157:H7 in T3 and T4, were lower ( $P<0.05$ ) than the control during storage time, reaching count reductions of 1.01 and 1.87 log CFU *E. coli* O157:H7/ g after 20 days of storage, respectively.

Similarly to these results, Cordeiro et al. [8] found reduction of approx. 2 log CFU/ g on the count of *E. coli* O 157:H7 in fermented sausages with different concentrations of mustard powder (AIT). Lemay et al. [7] observed a decrease of 2 log CFU/g on *E. coli* count in chilled chicken meat with AIT.

With regard to the instrumental color, there were no significant differences on L\* and b\* values among treatments. However, a\* (Table 4) and C\* (Table 5) values increased during the storage time in bulk sausages with essential oils.

T3 and T4, which received the highest doses of essential oils showed the highest a\* and C\* values and the lowest hue values during storage ( $P<0.05$ ).

Table 4. Instrumental color a\* values in bulk pork sausage with different combinations of garlic oil and allyl isothiocyanate (average  $\pm$  standard deviation)

Time (d)	Treatments				
	C	T1	T2	T3	T4
0	14,42 $\pm$ 0,57 <sup>a</sup>	14,42 $\pm$ 0,57 <sup>a</sup>	14,42 $\pm$ 0,57 <sup>a</sup>	14,42 $\pm$ 0,57 <sup>a</sup>	14,42 $\pm$ 0,57 <sup>a</sup>
5	18,08 $\pm$ 0,12 <sup>d</sup>	20,47 $\pm$ 0,57 <sup>bc</sup>	22,03 $\pm$ 0,11 <sup>a</sup>	21,24 $\pm$ 0,34 <sup>ab</sup>	20,37 $\pm$ 0,06 <sup>c</sup>
10	12,89 $\pm$ 1,30 <sup>b</sup>	19,84 $\pm$ 0,54 <sup>a</sup>	21,20 $\pm$ 0,30 <sup>a</sup>	20,83 $\pm$ 2,03 <sup>a</sup>	20,41 $\pm$ 1,36 <sup>a</sup>
15	13,52 $\pm$ 0,83 <sup>c</sup>	19,96 $\pm$ 1,21 <sup>b</sup>	21,38 $\pm$ 0,80 <sup>ab</sup>	23,94 $\pm$ 0,36 <sup>a</sup>	22,12 $\pm$ 1,59 <sup>ab</sup>
20	16,39 $\pm$ 0,37 <sup>b</sup>	15,96 $\pm$ 0,19 <sup>b</sup>	15,33 $\pm$ 0,67 <sup>b</sup>	19,52 $\pm$ 0,28 <sup>a</sup>	18,74 $\pm$ 0,71 <sup>a</sup>

C= control; AIT = allyl isothiocyanate; T1 = 125 ppm garlic oil + 125 ppm AIT, T2 = 250 ppm garlic oil + 125 ppm AIT; T3 = 125 ppm garlic oil + 250 ppm AIT, T4 = 250 ppm garlic oil + 250 ppm AIT. Different letters in the same line indicate significant differences among treatments ( $P<0.05$ ).

Table 5. Instrumental color C\* values in bulk pork sausage with different combinations of garlic oil and allyl isothiocyanate (average  $\pm$  standard deviation)

Time (d)	Treatments				
	C	T1	T2	T3	T4
0	18,12 $\pm$ 0,50 <sup>a</sup>	18,12 $\pm$ 0,50 <sup>a</sup>	18,12 $\pm$ 0,50 <sup>a</sup>	18,12 $\pm$ 0,50 <sup>a</sup>	18,12 $\pm$ 0,50 <sup>a</sup>
5	21,39 $\pm$ 0,07 <sup>b</sup>	23,46 $\pm$ 0,10 <sup>b</sup>	24,85 $\pm$ 0,11 <sup>a</sup>	23,39 $\pm$ 0,39 <sup>b</sup>	23,17 $\pm$ 0,09 <sup>b</sup>
10	17,38 $\pm$ 1,05 <sup>b</sup>	22,56 $\pm$ 0,62 <sup>a</sup>	23,81 $\pm$ 0,37 <sup>a</sup>	23,62 $\pm$ 1,43 <sup>a</sup>	23,37 $\pm$ 1,16 <sup>a</sup>
15	18,31 $\pm$ 1,18 <sup>c</sup>	23,03 $\pm$ 1,49 <sup>b</sup>	25,12 $\pm$ 1,13 <sup>ab</sup>	27,25 $\pm$ 0,53 <sup>a</sup>	25,40 $\pm$ 1,49 <sup>ab</sup>
20	19,53 $\pm$ 0,47 <sup>b</sup>	19,02 $\pm$ 0,23 <sup>b</sup>	19,37 $\pm$ 0,31 <sup>b</sup>	22,35 $\pm$ 0,38 <sup>a</sup>	21,65 $\pm$ 0,69 <sup>a</sup>

C= control; AIT = allyl isothiocyanate; T1 = 125 ppm garlic oil +125 ppm AIT, T2 = 250 ppm garlic oil + 125 ppm AIT; T3 = 125 ppm garlic oil + 250 ppm AIT, T4 = 250 ppm garlic oil + 250 ppm AIT. Different letters in the same line indicate significant differences among treatments ( $P < 0.05$ ).

Control sausages showed decrease on a\* and C\* values during storage and higher hue values compared to the sausages with essential oils. Decreased a\* values are usually associated to the gradual formation of metmyoglobin and, as a consequence, to meat discoloration [11]. Therefore, the addition of essential oils in pork sausages maintained the red color of meat, preventing discoloration during storage.

#### IV. CONCLUSION

The concentrations of essential oils of 125 ppm garlic oil + 250 ppm AIT or 250 ppm garlic oil + 250 ppm AIT were able to inhibit the growth of *E. coli* O157:H7 in bulk pork sausage. These concentrations were also able to maintain the red color of sausages during 20 days of storage. Further studies should be addressed to assess the impact of the antimicrobials on spoilage microorganisms and sensory characteristics of pork sausage.

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