

## EFFECT OF SODIUM LACTATE / SODIUM DIACETATE IN COMBINATION WITH SODIUM NITRITE ON PHYSIOCHEMICAL, MICROBIAL PROPERTIES AND SENSORY EVALUATION OF COW SAUSAGE

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### ABSTRACT

Sodium nitrite has been always considered as one of the common additives due to its antibacterial effects on *Clostridium botulinum* and meat products' color, however it produces cancer creating nitrosamine. Recently, organic acids and their salts such as lactates have been employed as antimicrobial compounds. Lactates also improve organoleptic properties including color, texture and taste and antioxidant properties. Sodium lactate causes to more reduction of anaerobic spore former bacteria than nitrite, inhibits botulin produced by *Clostridium botulinum*. Sodium lactate produces a permanent reddish pink color through reduction of deoxymyoglobin and producing deoxymyoglobin. In this study, the decrease of sodium nitrite amount from 120ppm to 15ppm by adding sodium lactate / sodium diacetate led to achieve an acceptable product. The best results revealed through adding 3.0625% of sodium lactate / sodium diacetate in combination with 30ppm sodium nitrite. Results also exhibited more reduction of pathogens' growth than nitrite, enhanced flavor slightly, but unable to produce reddish pink color as produced by nitrite. Results also exhibited that sodium lactate / diacetate cause to retard in microbial growth, reducing chemical change, enhance sensory properties, partially improvement in taste and texture. Although inappropriate color demonstrated sodium lactate / diacetate's inability in red pink color production in 4th sample (contains 15 ppm nitrite), its synergy effect in combination with sodium nitrite on nitroso myoglobin production has been proven, led to sodium nitrite reduction in sausages.

**Keywords:** Sodium lactate; sodium acetate; physiochemical properties; microbial properties; cow sausage

### INTRODUCTION

Currently, demanding growth of fast food consuming has been increased due to their easy preparing. While most of microorganisms lives in low heated treatment, reproducing of microorganisms during storage lead to food spoilage and health risk. Nitrite and nitrate have positive effects on products' color and suitable antimicrobial effects on *Clostridium botulinum*, however they produce cancer creating nitrosamine. Purchasing meat products base on color exhibits the important role of this factor as freshness and healthiness. Changing this factor can consider as a sign of the decrease of consumer acceptance and shelf life (Bingöl and Boostan, 2007). Sodium lactate decreases M.O growth and pH, maintains taste in 7 - 10 days in comparison with control, inhibits *Clostridium botulinum* and *Listeria monocytogenes* growth as well (Brewer et al., 1991). Adding of antimicrobial and antioxidant compounds like sodium lactate (Choi and Chin, 2003) to increase shelf life is one way to replace nitrite in meat products. Sodium lactate which is an associated acid penetrates across from microbial membrane, acidifies inner part of cell, but unable to produce red color like nitrite (Choi and Chin, 2003). While lactate uses as an antimicrobial compound in meat products, there are a few studies on lactate's effect on color development. This study aims to investigate the affection on nitroso

myoglobin, minimizing sodium nitrite in cow sausage in order to increase shelf life, keeping sensory properties and products' color. The results come from measuring of bacterial growth in storing time, color assessment, oxidative rancidity and sensory evaluation (Hunter and Segel, 1973).

### MATERIAL AND METHODOLOGY

#### Production procedure

Frozen beefsteak minced into small pieces by Magurit guillotine, extruded by KramerGrebe extruder (model ww 160, 3 mm mesh), mixed by salt, sodium nitrite, sodium lactate / diacetate in Seydelmann cutter model K120 Ras 60090. This method causes to a better extraction and solution of myofibril protein which creates an emulsion like structure and sticky membrane around meat pieces. After adding sodium polyphosphate, half of ice and water added, then filler compounds after 30s paste cutting, finally the rest of ice added, paste cutting continued to produce a homogenous mixture. Table 1 shows the sodium acetate / diacetate in 4 samples.

Cow sausage ingredients are including 12 kg of beefsteak meat with 10% fat, soybean oil 1.6 kg, non-skinned fresh garlic 0.8 kg, wheat flour 0.8 kg, ice 3 kg, gluten 1 kg, potato starch 0.2 kg, season 0.06 kg,

**Table 1** Sodium lactate/diacetate and nitrite sodium in 4 produced sample.

Samples	Sodium chloride (gr)	Sodium lactate / diacetate (%)	Sodium nitrite (ppm)
Sample 1	0.3	3.0625	15
Sample 2	0.27	2.625	30
Sample 3	0.2475	1.75	60
Sample 4	0.239	0	120

0.08 kg of sodium poly phosphate 60%, ascorbic acid 0.02 kg, soybean protein isolate 0.2 kg.

And then paste carried to filler Vemag model Robot DP10C, filled in 5 layers poly amid casings produced by Mashahad Arta Co (diameter 2.7 cm, length 30 cm), cooked at 75 °C for 45 min in 6 wagons cooking room (produced by wimeg company in dimension 3 × 2 m, finally kept at 5 ±1 °C to evaluate their shelf life.

**Total count**

Plate count agar (Fluka) prepared, sterilized in autoclave at 121 °C for 15 min, put in water bath at 47 °C, then one ringer Merk tablet dissolved in 500 ml distilled water, autoclaved at 121 °C 15 min.

Samples extruded to small pieces by steel and sterilized extruder (in oven at 180 - 200 °C for 2 hr). 10 g of sample added to 90 mL ringer solution, shook and kept in station for 15 min, 9 mL of ringer sterilized solution poured in test tube (in oven at 180 - 200 °C for 2 hr), adding 1 mL of last dilution achieved 0.01 dilution. To achieve 10<sup>-5</sup> follow this method again.

Samples carried to plates by sterile pipet after dilution, 12-15 ml of PCA added to plates, shook to produce a homogenous mixture, after setting put and kept in incubator upside-down at 30 ±1 °C for 72 ±3 hr. Consecutive plates contain 15-300 colonies counted and the colony amount calculated based on the following formula:

$$N = \frac{\sum C}{V(n_1 + 0/1n_2)d}$$

In which:

- ∑C: Sum of selected colonies
- V: Injected volume in each pipet
- n<sub>1</sub>: Counted plates in 1st dilution
- n<sub>2</sub>: Counted plates in 2nd dilution
- d: Dilution coefficient base on 1st selected dilution

**Color measurement**

Cow sausage's color Measured using Hunter lab Color flex spectrophotometer model No45/0, s.ncx2547. The average of triplicate color measurements was recorded, and the results were expressed in terms of lightness (L\*), redness (a\*), and yellowness (b\*). 3 Random sausage rolls selected in each replicate, cut their head and tail, filled in cell (D=6.35 cm), putting black cover on it, light radiated in 6 different and random angles, recorded the resultant N.Os.

**Texture profile analysis**

A texture analyzing TPA Lloyd model TA Plus MTM S/NO. 108059 Version 3/64 Issue 1 and software NEXYGENPlus Materials Testing and Data Analysis Software, version 2.1, copyright 2009, Ametek, inc. was used to conducted to texture profile analysis. Samples

placed in ambient temperature 15 min at 25 mL, cut into pieces (diameter 2.7 cm and height 2 cm) placed under a cylindrical probe. Samples compressed by a 3 inches aluminum sheet twice to 50% of their original height (load cell and rate were 500 N and 1 mm/s respectively), factors including hardness, stickiness, springiness, brittleness, deformation force, rough ness and sticky force measured (Yang et al., 2007).

**Thiobarbitoric acid measurement**

50 - 200 mg of sample weighed, poured in Volumetric flask 25 mL, dissolved by 1-butanol and reached to volume, 5mL of solution carried to test tube by pipet, indicator added, shook and placed in thermostatic bath at 95 °C for 2 hr, then cooled by cool water for 10 min to room temperature, its absorption recorded using spectrophotometer model shimadzo UV 1700 in 530 nm wavelength, TBA calculates based on the following formula:

$$TBA\ value = \frac{50 \times (A - B)}{m}$$

In which:

- A: Absorption of experimented solution
  - B: Absorption of control solution
  - M: Mass at mg
- And number 50 is correction factor when volumetric flask is 25 mL and cell width was 10 mm.

**2.5 Cow sausage sensory evaluation**

A six group of penalist described sensory evaluation including flavor, taste, appearance, texture. Skilled panelist quantifying these factors, then quality factors determined based on a 15 cm accurate scale (Table 2).

**Chemical properties analysis**

**Protein percent measurement**

1 g of sample weighed on paper, poured on a 25 mL digestion flask, concentrated sulfuric acid added, 8 g catalyzer mixture including 96% potassium sulfate (7.68 g), 3.5% copper sulfate (0.28 g), 0.5% selenium oxide (0.04 g), snappy flask contains sodium hydroxide 30% placed on digestion flask, heated 15min not to foam while observing green color, experiment continued 1 hr. (According to Iran National Standard N. O. 924). Compounds in flask carried to distillation flask by 200-250 mL distilled water, some glass Perl added, placed under a condenser which contains 50 mL buric acid 4% and some droplets of bromochrosol green poured in it, then flask heated, 70 - 80 mL sodium hydroxide 5% poured by funnel, flask heated to collected 200 - 250 mL distilled solution containing ammoniac, titrated by chloridric acid and protein calculated by the following formula:

$$\%P = \frac{V \times 0/000114 \times 100 \times 6/25}{m}$$

**Table 2** Evaluated factors of formulated cow sausages

Taste	Mouth fell	Basic flavor	Smell	Appearance	Traits
chemical sourness season meat cow taste unfavorable smell and taste	Strength frangibility juiciness brittleness	Sourness bitterness	Aroma meat rancidity	Color homogenous	Traits features

**Table 3** Analysis of 4 cow sausage produced samples

samples	Features (%)			
	humid	fat	ash	protein
Sample 1	62.80	11.42	2.71	16.41
Sample 2	62.93	11.50	2.72	16.52
Sample 3	62.70	11.41	2.72	16.90
Sample 4	62.75	11.44	2.68	16.41

**pH measurement**

pH meter calibrated by buffers N.O 4 and 7. Sausages extruded twice, mixed and pH measured.

**Ash measurement**

Crucible heated in furnace at 550 ±25 °C for 20 min, cooled in desiccator and weighed, then 1 g of sample poured in it, heated on flame up to no smoke observed, heated in furnace at 550 ±25 °C for 4 - 6 hr, cooled in desiccator and weighed, ash calculates based on the following formulation (in accordance with national Iran standard N. O. 1028):

$$\text{Ash \%} = \frac{m_2 - m_0}{m_1 - m_0} \times 100$$

In which:

- m2: Contain ash weight
- m1: Weight of crucible contain sample
- m0: Wight of empty crucible

**Humidity measurement**

Plate contain lab sand dried in oven at 103 ±2 °C for 60 - 90 min, cooled in desiccator and weighed. 5 g of sample carried to plate, mixed by sand and ethanol uniformly, dried at the same conditions cooled in desiccator and weighed (in accordance with national Iran standard N. O. 745).

**Fat measurement**

5 g of sample weighed, poured in flask, heated by 50 mL hydrochloric acid 50% for 30 min (digestion stage), cooled, filtered through filter paper and washed by distilled water up to no acid trace observed in out water, then filter paper dried in oven at 103 ±2 °C. Soxhelt flask also dried at the same condition for 1 hr, placed in desiccator, cooled and weighed.

Then filter paper rolled, placed in extraction cartouche, fat absorbed by hexane inoculated cotton to cartouche, then cartouche placed in extraction part and hexane added.

Soxhelt set up and heated by electric heater, this continued to 8 - 10 hr., cartouche removed and the most

part of hexane removed, then separated and solvent vapored by water bath, put in oven for 90 min at 103 ±2 °C dried and cooled in desiccator and weighed.

**Statistical Analysis**

Statistical analysis performed based on complete randomized block design with factorial and Minitab 16th software. Average comparison performed by Duncan experiment and SPSS 20th software ( $\alpha < 0.05$ ).

**RESULTS AND DISCUSSION**

**Basic experiments**

To insure that all produced samples are similar in chemical properties, 4 produced samples tested (2 times) in 1st week, with respect to no significant differences ( $p < 0.05$ ) between samples, other experiments tested (Table 3).

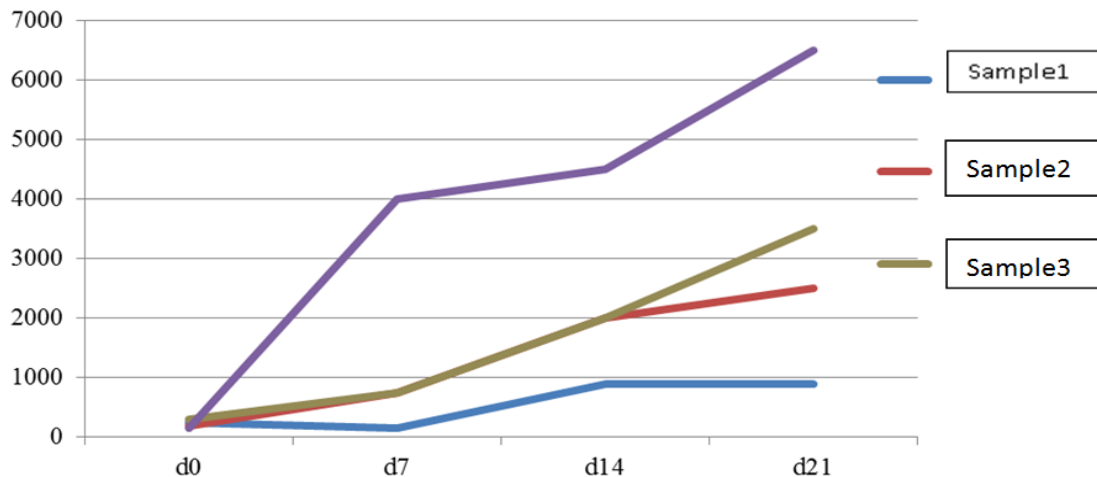
**Microbial experiment results**

**Total microbial count**

Sodium lactate is a salt which acts as associated acid, passes microbial cell membrane, and acidifies inner part of cell (Carpenter and Broadbent, 2009). Denaturation can lead to quick pH reduction which causes to cell death (Lamkey et al., 1991). No significant differences observed in 1st week, sample 4th which contained 120 ppm nitrite displayed more microbial count reveals that adding sodium lactate / diacetate has been better proficiency in preventing microbial growth in other samples, the best result observed in sample 1 with respect to its slowest microbial growth between samples. No significant differences observed in 1st week, sample 4th which contained 120 ppm nitrite displayed more microbial count reveals that adding sodium lactate / diacetate has been better proficiency in preventing microbial growth in other samples, the best result observed in sample 1 with respect to its slowest microbial growth between samples (Table 4).

**Table 4** Cow sausage total count (CFU/g) during 21 days storing time at 5 °C.

samples	Days			
	0	7	14	21
<b>Sample 1</b>	$2.5 \times 10^2$ a,A	$1.5 \times 10^2$ a,A	$9 \times 10^2$ a,B	$9 \times 10^2$ a,B
<b>Sample 2</b>	$2 \times 10^2$ a,A	$7.5 \times 10^2$ a,A	$2 \times 10^3$ b,B	$2.5 \times 10^3$ a,b,B
<b>Sample 3</b>	$3 \times 10^2$ a,A	$7.5 \times 10^2$ a,A,B	$2 \times 10^3$ b,C	$3.5 \times 10^3$ b,D
<b>Sample 4</b>	$1.5 \times 10^2$ a,A	$4 \times 10^3$ b,B	$4.5 \times 10^3$ c,B	$6.5 \times 10^3$ c,C



**Figure 1** Cow sausage total count (CFU/g) during 21 days storing time at 5 °C.

Adding 3.0625% sodium lactate / diacetate in combination with 30 ppm nitrite to 2nd sample reduced microbial growth more than 4th sample and displayed better antimicrobial effects, no changes in sensory properties, improved quality, however its only defect related not to create red pink color which observed by increasing sodium lactate / sodium diacetate % and reducing sodium nitrite to 15 ppm in sample 1 (Figure 1). High level amounts of lactate can change pirovate reaction to lactate, prevents anaerobic growth, therefore adding sodium lactate causes to reduce microbial growth by aw reduction (Meng and Genigeorgis, 1993). Number of *Clostridium botulinum* trace found, more reduction of anaerobic spore former bacteria number than nitrite.

Findings report that species including *Debaryomyces hunseni* and *Candida* separated from meat products (Houtsma et al., 1993) can tolerate sodium lactate, this is 10 times stronger in separated yeasts, while sodium lactate displays no tangible effect on yeast, postpones mold growth 30 days (Bingöl and Bostan, 2007).

**Staphylococcus aureus and Clostridium perfringenes count**

None of the samples displays these bacteria, no assumption can present about them, however in other researches inhibiting effects of sodium lactate / diacetate against former bacteria have been reported.

**Chemical experiment results**

**pH measurement**

Buffering capacity in meat seems to be effective on keeping low pH of meat, the same results in comparison of other research, no significant differences between 1 and

3% sodium lactate / diacetate (Table 5) in pH changing. (Gonzalez-Fandos et al., 2009).

Sodium lactate / diacetate keep pH during storing time due to its buffer capacity.

Number of significant differences observed in 4 samples during 21 days which showed the pH stability during storing at 5 °C, the same result in comparison with Brewer et al. (1991) in which adding 2 - 3% sodium lactate in pig sausage caused to postpone pH reduction, besides findings about keeping pH by adding 3% sodium lactate during 30 days storing at 20 °C, additionally in another study keeping pH in 4 - 12 weeks storing in (Figure 2).

**Oxidative rancidity measurement (TBA index)**

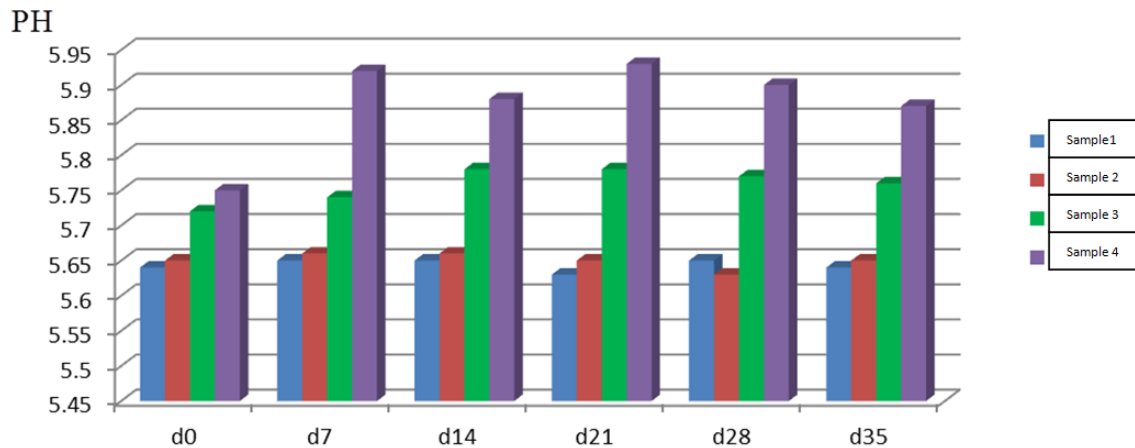
Oxidative rancidity is known as the second most important factor in quality reduction in meat products. Rancidity occurs through unsaturated fatty acid, oxygen or other oxidizing compounds such as iron which is influenced by peroxidant compounds, heat and light (Cheng et al., 2007). Lactates minimize fatty acids reduction that effects on color stability as fat and myoglobin oxidation are dependent reactions lead to reduction of meat's color lifetime (Mancini et al., 2009). Thiobarbituric acid showed no significant differences in the beginning of storing time (Table 6), however 3rd sample (containing 60 ppm sodium nitrite and 1.75% sodium lactate / diacetate) and 1st sample (containing 15 ppm sodium nitrite and 3.0625% sodium lactate / diacetate) displayed the most and the least thiobarbituric acid respectively. Results also showed that sodium lactate / diacetate has more prominence than sodium nitrite (120 ppm) in 4th sample.

**Table 5** pH results during 35 days of storing at 5 °C.

Samples	Days					
	0	7	14	21	28	35
<b>Sample 1</b>	5.64	5.65	5.65	5.63	5.65	5.64
<b>Sample 2</b>	5.65	5.66	5.66	5.65	5.63	5.61
<b>Sample 3</b>	5.72	5.74	5.78	5.78	5.77	5.76
<b>Sample 4</b>	5.75	5.92	5.88	5.93	5.90	5.87

**Table 6** TBA amounts of sausages during 21 days of storing at 5 °C.

Samples	Days			
	0	7	14	21
<b>Sample 1</b>	0.051 <sup>a,B</sup>	0.059 <sup>b,C</sup>	0.08 <sup>c,D</sup>	0.031 <sup>b,A</sup>
<b>Sample 2</b>	0.049 <sup>a,A</sup>	0.059 <sup>c,B</sup>	0.11 <sup>d,D</sup>	0.082 <sup>d,C</sup>
<b>Sample 3</b>	0.049 <sup>a,D</sup>	0.069 <sup>c,C</sup>	0.036 <sup>b,B</sup>	0.015 <sup>a,A</sup>
<b>Sample 4</b>	0.053 <sup>a,B,C</sup>	0.051 <sup>a,B</sup>	0.028 <sup>a,A</sup>	0.059 <sup>c,C</sup>



**Figure 2** pH results during 35 days of storing at 5 °C.

### Sensory evaluation

No significant differences observed between 2nd and 3rd sample which contained 3.02652% and 2.625% sodium lactate / diacetate respectively in comparison with 4th sample contained 120 ppm sodium nitrite thus the only decision made by panelists about quality reduction related to 1st sample's color.

### Texture analysis

Increasing sodium lactate / diacetate reduced texture properties including springiness, hardness, roughness and stickiness in samples, however these factors were more in 4th sample which revealed the frangibility property by adding sodium lactate / diacetate in other samples, clearly showed in 1st and 1nd samples containing 2.625% and 3.0625% respectively. Changing in samples' tecture and color which contained 0.6% and 1.2% sodium lactate / diacetate observed after 45 and 60 days (Table 7) (in agrrement with **Bingöl and Bostan (2007)**).

### Color measurement

Nitrosmyoglobuline which is the most important meat pigment transforms to a pink pigment called nitrosyl miocorgen. L factor varied during storage. While b factor increased in 4th sample, decreased in other samples, yet 2nd sample's "a index" was more than for in 1st and to the end of 21st storing day, but 1st sample (contains 15 ppm sodium nitrite, 3.6025% sodium lactate / diacetate) displayed the least "a index". These results were in agreement with **Brooke et al. (2011)** results (Table 8).

Adding lactate reduced rest nitrite (base on **Brooke et al., 2011** results), both experiments support this assumption that lactate produce NADH, which reduced metmyoglobuline to deoxmyoglobuline or lactate myoglobuline reaction. More lactate also reduces myoglobuline, subsequently react nitrite produces more nitric oxide and reduces nitrite concentration (**Brooke et al., 2011**).

**Table 7** Sensory evaluation results of sausages during 21 days of storing at 5 °C.

Property	Sample	Days		Property	Sample	Days	
		0	21			0	21
Basic taste	Sample 1	4.25 <sup>a,A</sup>	<sup>a,A</sup> 4.25	Total quality	Sample 1		
Sourness	Sample 2	3 <sup>b,A</sup>	<sup>b,A</sup> 3		Sample 2		
	Sample 3	<sup>b,A</sup> 3	<sup>b,A</sup> 2.75		Sample 3		
	Sample 4	2 <sup>c,A</sup>	<sup>c,A</sup> 2		Sample 4		
Saltiness	Sample 1	6 <sup>a,A</sup>	<sup>a,A</sup> 5.25	Appearance			
	Sample 2	<sup>a,A</sup> 5.25	<sup>a,A</sup> 4.75	Color homogeneity	Sample 1		
	Sample 3	4.5 <sup>b,A</sup>	4 <sup>b,A</sup>		Sample 2		
	Sample 4	4.5 <sup>b,A</sup>	<sup>b,A</sup> 4.5		Sample 3		
Taste and smell	Sample 1	13.75 <sup>a,A</sup>	<sup>a,B</sup> 12.5		Sample 4		
Meat flavor	Sample 2	13.25 <sup>a,b,A</sup>	<sup>a,b,B</sup> 11.5	Mouth feel			
	Sample 3	11.75 <sup>b,A</sup>	<sup>b,B</sup> 10.75	Hardness	Sample 1	8.25 <sup>a,A</sup>	6.75 <sup>a,A</sup>
	Sample 4	10.25 <sup>c,A</sup>	<sup>c,B</sup> 9.25		Sample 2	10 <sup>b,A</sup>	<sup>b,A</sup> 9.75
Fat complex	Sample 1	<sup>a,A</sup> 6.25	<sup>a,A</sup> 7.5		Sample 3	11.5 <sup>c,A</sup>	<sup>c,A</sup> 12.75
	Sample 2	<sup>a,A</sup> 4.75	<sup>a,A</sup> 5.5		Sample 4	13.75 <sup>d,a</sup>	<sup>d,a</sup> 13.5
	Sample 3	<sup>a,A</sup> 4.75	<sup>a,A</sup> 4.25	Frangibility	Sample 1	13 <sup>a,A</sup>	<sup>a,A</sup> 13
	Sample 4	<sup>a,A</sup> 3.5	<sup>a,A</sup> 3.25		Sample 2	11.75 <sup>b,A</sup>	<sup>b,A</sup> 10.5
Meat Taste	Sample 1	14 <sup>a,A</sup>	<sup>a,A</sup> 12.5		Sample 3	<sup>b,A</sup> 11.75	<sup>b,A</sup> 10.25
	Sample 2	<sup>a,A</sup> 13	<sup>a,A</sup> 11.5		Sample 4	9.5 <sup>c,A</sup>	<sup>c,A</sup> 7.25
	Sample 3	10.25 <sup>b,A</sup>	<sup>b,A</sup> 10.5	Juiciness	Sample 1	12.5 <sup>a,A</sup>	<sup>a,A</sup> 13.75
	Sample 4	<sup>b,A</sup> 9	<sup>b,A</sup> 9.75		Sample 2	11.75 <sup>b,A</sup>	<sup>b,A</sup> 10.75
Brittleness	Sample 1	8 <sup>a,A</sup>	<sup>a,A</sup> 6.75		Sample 3	<sup>b,A</sup> 10.5	<sup>b,A</sup> 9.5
	Sample 2	9 <sup>b,A</sup>	<sup>b,A</sup> 8.75		Sample 4	<sup>c,A</sup> 7.5	<sup>c,A</sup> 6.5
	Sample 3	10 <sup>c,A</sup>	<sup>c,A</sup> 10				
	Sample 4	12.75 <sup>d,A</sup>	<sup>d,A</sup> 13				

a,b,c,d : Least squares means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

A,B,C,D : Least squares means with different superscripts in the same row differ significantly ( $p < 0.05$ ).

Nitrite reaction rate influenced by meat properties, surrounded conditions which change processing conditions such as adding sodium erisorbate or sodium ascorbate and pH reducing products, dramatically increase nitrite reduction to nitric oxide (Honikel, 2008).

It can assume that adding lactate increase reduction capacity of myoglobuline, more producing deoxymyoglobuline, therefore more rest nitrite reduction and more nitric oxide production predicts (Brooke et al., 2011).

## CONCLUSION

Results indicated that sodium lactate / diacetate cause to: retard in microbial growth, reducing chemical changes, enhance sensory properties and partially improve taste and texture. Although inappropriate color demonstrated sodium lactate / diacetate's inability in red pink color production in 4th sample (contains 15 ppm nitrite), its synergy effect in combination with sodium nitrite on nitroso myoglobuline production has been proven and led to sodium nitrite reduction in sausages. Using these cheap

materials can consider as an evolution in meat industry, in other words more studies need to be done on suitable additives in order to develop products, because of more complication in this field related to animal genetics, chemical structure of muscles, pre and post mortem condition, manufacturing, packaging distribution, storing and etc.

With respect to the results of this study, future studies can focus on factors effecting meat properties in combination with sodium lactate / sodium diacetate, removing nitrite by replacing it with other compounds and other factors affecting nitrosomyoglobuline formation. Sodium lactate / diacetate postpones microbial growth depends on its concentration, cause no pH change during storing but effects on microbial growth. This study revealed that not only adding Sodium lactate / diacetate improve microbial quality depends on its concentration, but improve shelf life with better effects than sodium nitrite, however meat science must focus on development in the field of color improvement in relation with no sodium nitrite consuming. Future trends can answer to these questions.

Table 8 TPA results of sausages during 21 days of storing at 5 °C.

Index	sample	Days			
		0	7	14	21
Hardness	Sample 1	6.68 <sup>a,A</sup>	6.44 <sup>a,A</sup>	6.18 <sup>a,A</sup>	6.66 <sup>a,A</sup>
	Sample 2	6.78 <sup>a,A</sup>	6.36 <sup>a,A</sup>	6.31 <sup>a,A</sup>	6.43 <sup>a,A</sup>
	Sample 3	10.68 <sup>b,A</sup>	10.35 <sup>a,A</sup>	10.25 <sup>a,A</sup>	10.48 <sup>a,A</sup>
	Sample 4	11.39 <sup>c,A</sup>	11.39 <sup>a,A</sup>	11.51 <sup>a,A</sup>	11.22 <sup>a,A</sup>
SEM		0.113	0.113	0.113	0.113
Stickiness	Sample 1	0.149 <sup>a,A</sup>	0.153 <sup>a,A</sup>	0.149 <sup>a,A</sup>	0.155 <sup>a,A</sup>
	Sample 2	0.166 <sup>b,A</sup>	0.164 <sup>b,A</sup>	0.145 <sup>b,A</sup>	0.162 <sup>b,A</sup>
	Sample 3	0.213 <sup>c,A</sup>	0.213 <sup>c,A</sup>	0.206 <sup>c,A</sup>	0.216 <sup>c,A</sup>
	Sample 4	0.233 <sup>d,A</sup>	0.233 <sup>d,A</sup>	0.199 <sup>d,A</sup>	0.232 <sup>d,A</sup>
SEM		0.871	0.871	0.871	0.871
Springiness	Sample 1	5.80 <sup>a,A</sup>	5.53 <sup>a,A</sup>	5.61 <sup>a,A</sup>	5.76 <sup>a,A</sup>
	Sample 2	6.35 <sup>a,A</sup>	5.56 <sup>a,A</sup>	5.54 <sup>a,A</sup>	5.99 <sup>a,A</sup>
	Sample 3	7.17 <sup>b,A</sup>	7.04 <sup>b,A</sup>	7.22 <sup>b,A</sup>	7.36 <sup>b,A</sup>
	Sample 4	7.46 <sup>b,A</sup>	7.46 <sup>b,A</sup>	7.25 <sup>b,A</sup>	7.37 <sup>b,A</sup>
SEM		0.201	0.201	0.201	0.201
Brittleness	Sample 1	5.56 <sup>a,A</sup>	5.47 <sup>a,A</sup>	5.19 <sup>a,B</sup>	5.97 <sup>a,A</sup>
	Sample 2	6.27 <sup>a,A</sup>	5.20 <sup>a,A</sup>	5.10 <sup>a,A</sup>	6.27 <sup>a,A</sup>
	Sample 3	15.50 <sup>b,A</sup>	15.59 <sup>b,A</sup>	15.33 <sup>b,A</sup>	16.75 <sup>b,A</sup>
	Sample 4	19.82 <sup>c,A</sup>	19.82 <sup>c,A</sup>	19.61 <sup>c,A</sup>	19.20 <sup>c,A</sup>
SEM		0.542 <sup>a</sup>	0.542	0.542	0.542
Refract force	Sample 1	0.416 <sup>a,A</sup>	0.419 <sup>a,A</sup>	0.419 <sup>a,A</sup>	0.421 <sup>a,A</sup>
	Sample 2	0.417 <sup>a,A</sup>	0.413 <sup>a,A</sup>	0.419 <sup>a,A</sup>	0.417 <sup>a,A</sup>
	Sample 3	0.434 <sup>a,A</sup>	0.422 <sup>a,A</sup>	0.425 <sup>a,A</sup>	0.422 <sup>a,A</sup>
	Sample 4	0.419 <sup>a,A</sup>	0.419 <sup>a,A</sup>	0.422 <sup>a,A</sup>	0.417 <sup>a,A</sup>
SEM		0.838 <sup>a</sup>	0.838 <sup>a,A</sup>	0.838	0.838
Rigidity	Sample 1	1.21 <sup>a,A</sup>	1.12 <sup>a,A</sup>	1.14 <sup>a,A</sup>	1.18 <sup>a,A</sup>
	Sample 2	1.14 <sup>a,A</sup>	1.14 <sup>a,A</sup>	1.13 <sup>a,A</sup>	1.13 <sup>a,A</sup>
	Sample 3	1.73 <sup>b,A</sup>	1.66 <sup>b,A</sup>	1.74 <sup>b,A</sup>	1.73 <sup>b,A</sup>
	Sample 4	1.94 <sup>b,A</sup>	1.94 <sup>b,A</sup>	1.98 <sup>b,A</sup>	1.85 <sup>b,A</sup>
SEM		0.745	0.745	0.745	0.745

a,b,c,d : Least squares means with different superscripts in the same row differ significantly ( $p < 0.05$ )  
A,B,C,D : Least squares means with different superscripts in the same row differ significantly ( $p < 0.05$ )  
ABBREVIATION :SEM Standard Error Mean

Table 9 TPA results of color sausages during 21 days of storing at 5 °C.

Index	Sample	Days			
		0	7	14	21
L	Sample1	54.5 <sup>d,A</sup>	<sup>d,B</sup> 54.8	<sup>d,C</sup> 55.4	<sup>d,D</sup> 55.7
	Sample 2	53.4 <sup>c,A</sup>	<sup>c,B</sup> 53.9	<sup>c,C</sup> 54.1	<sup>c,D</sup> 54.8
	Sample 3	52.7 <sup>a,A</sup>	<sup>a,B</sup> 53.3	<sup>a,B</sup> 53.6	<sup>a,B</sup> 53.6
	Sample 4	<sup>b,A</sup> 53.1	<sup>b,B</sup> 53.6	<sup>b,C</sup> 53.8	<sup>b,D</sup> 54.2
SEM		0.0252	0.0252	0.0252	0.0252
	Sample1	<sup>a,D</sup> 6.9	<sup>a,C</sup> 6.7	<sup>a,B</sup> 6.2	<sup>a,A</sup> 6
	Sample 2	<sup>d,D</sup> 12.3	<sup>d,C</sup> 12.2	<sup>d,B</sup> 12.1	<sup>d,A</sup> 11.9
	Sample 3	<sup>b,A</sup> 11.6	<sup>c,B</sup> 11.8	<sup>c,C</sup> 12	<sup>c,A</sup> 11.6
	Sample 4	<sup>c,C</sup> 11.8	<sup>b,A</sup> 11	<sup>b,B</sup> 11.1	<sup>b,B</sup> 11.1
SEM		0.0711	0.0711	0.0711	0.0711
	Sample1	<sup>d,A</sup> 12.2	<sup>d,B</sup> 12.5	<sup>d,D</sup> 12.8	<sup>c,C</sup> 12.6
	Sample 2	<sup>b,C</sup> 11.5	<sup>a,B</sup> 11.4	<sup>a,C</sup> 11.5	<sup>a,A</sup> 11.3
	Sample 3	<sup>c,B</sup> 12.1	<sup>b,A</sup> 11.8	<sup>b,A</sup> 11.8	<sup>b,A</sup> 11.8
	Sample 4	<sup>a,A</sup> 11.4	<sup>c,B</sup> 12.2	<sup>c,C</sup> 12.5	<sup>c,D</sup> 12.6
SEM		0.0115	0.0115	0.0115	0.0115

a, b, c, d: Least squares means with different superscripts in the same row differ significantly ( $p < 0.05$ )  
A, B, C, D: Least squares means with different superscripts in the same row differ significantly ( $p < 0.05$ )  
ABBREVIATION: SEM Standard Error Mean

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