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 The objective of this study was to determine the effect of using vegetable juice powder on microbiological and physico-chemical characteristics during the manufacture process of a dry fermented sausage "chorizo". To achieve this aim four batches of small caliber fermented sausages were manufactured: 1- Control batch: without nitrates and nitrites added, 2- batch 1: with nitrates and nitrites added, 3- batch 2: with vegetable juice powder, and 4batch 3: with vegetable juice powder and with starter cultures. Samples of each batch were analysed after 0, 5, 16 and 26 days of processing. Microbiological and physicochemical parameters were tested. In general, all microbiological counts increased during the manufacture process of the four batches of "chorizo" and no differences or slight differences were found for the microorganisms studied in the final product. In all days of processing, the sausages manufactured with nitrite and nitrate added (batch 1) presented the highest values for the nitrate and nitrite content and no differences (p>0.05) were found between the others three sausages batches. For that reason further studies are neccesary carried out to optimize the use of vegetable juice as sources of nitrates and nitrites in dry fermented meat products.

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Index Terms—chorizo, fermented sausages, nitrites, nitrates, vegetable juice powder.

# I. INTRODUCTION

 $\mathbf{T}$  he manufacture process of the cured meat products is characterized by the addition of cured agents, nitrate and/or nitrite, which improve the microbiological safety and contribute to the sensory characteristics. Nitrate is used as a source of nitrite since it is reduced to nitrite by nitrate reductase under anaerobic conditions [1], [6]. Nitrite is the active agent in the curing mixture and its role in cured meat products can be summarised as: a) characteristic formation of cured meat colour, b) inhibition of spoilage and pathogenic bacteria; c) contribution to typical flavour development and c) contribution to oxidative stability of meat [4], [5]. An alternative to the use of nitrite in cured meat products is to use vegetable juice powder (with nitrate as a natural resource) and a starter culture with nitrate reductase activity.

The objective of this study was to determine the effect of using vegetable juice powder on microbiological and physicochemical characteristics during the manufacture process of a dry fermented sausage "chorizo".

## II. MATERIALS AND METHODS

To achieve this aim four batches of small caliber fermented sausages were manufactured: *I*- Control batch: without nitrates and nitrites added, *2*- batch 1: with nitrates and nitrites added, *3*- batch 2: with vegetable juice powder, and 4- batch 3: with vegetable juice powder and with starter cultures.

All the sausages were manufactured the same day, using the same technology and according to a traditional formulation, which consisted of 75% pork meat and 25% pork backfat. Lean pork meat and pork backfat were minced (P-32 FUERPLA, Valencia, Spain) to a particle size of about 8 mm and subsequently mixed in a vacuum mixer (A-85 FUERPLA, Valencia, Spain) with the corresponding ingredients (table 1). The different sausage mixtures were stuffed into casings (40-45 mm Ø). All sausages were fermented in a drying chamber (Hermekit, Cenfrio, Spain) at 15°C and 90-100% relative humidity (RH) for 18 h, 22-23°C and 90 % RH for 48 h, at 14-15°C and 80-90% RH for 10 days. Then the RH was slowly reduced to 75% until the end of the ripening process (a total of 26 days).

Samples of each batch were taken during the following phases: initial sausage mixture (day 0), after fermentation (day 5) and dry curing (days 16 and 26) to carry out the different analysis.

Microbiological analysis. The samples were analysed for aerobic mesophilic bacteria (3M Petrifilm Aerobic Count Plate (3M, Madrid, Spain) at 30°C for 72 h), *Enterobacteriaceae* (3M Petrifilm Enterobacteriaceae Count Plate (3M, Madrid, Spain) at 37°C for 24h), lactic acid bacteria (LAB) (MRS Agar (Scharlau, Spain) at 30°C for 48 h), and *Micrococcaceae* (MSA Agar (Scharlau, Spain) at 37°C for 48 h).

**Physicochemical analysis.** pH was determined with a Crison 2001 pH meter (Crison Instruments S.A., Barcelona, Spain) equipped with a punction electrode. Water activity (a<sub>w</sub>) was measured using a Decagon CX-2 AQUALAB hygrometer (Decagon Devices Inc., Pullman, WA, USA). Nitrate and nitrite content were determined according to the ISO methods 3091:1975 [3] and 2918:1975 [2] respectively.

**Statistical analysis.** Data sets were statistically analysed using one-way variance analysis (ANOVA) in order to determine any significant differences during the manufacture process in each batch and between the four different batches in each time. The means were separated by Tukey-honest significant difference test at 5% level. Data analyses were conducted using STATISTICA 6.0 statistical package.

#### III. RESULTS AND DISCUSSION

The results of the microbiological analysis are showed in the table 2. In general, all microbiological counts increased during the manufacture process of the four batches of "chorizo". At day 0, the sausages of the batch 3 presented higher counts for aerobic mesophilic bacteria, lactic acid bacteria due to the addition of starter culture in the manufacture of these sausages. In the final product (26 days), no differences or slight differences between batches were found in the counts obtained for the microorganims studied. The typical microflora values obtained were usual for this product in all batches.

The weight loss (table 3) increased during the days of processing in all batches up to 40%. No differences (p>0.05) were found between batches in all samples times.

During the manufacture process the pH values (table 3) were reduced in all groups probably due to the activity of lactic acid bacteria. The results of  $a_{\rm w}$  (table 3) showed a decrease during the days of processing due to the drying process. In the final product, the sausages control (without nitrate and nitrite added) presented the highest pH and  $a_{\rm w}$  values.

The nitrate and nitrite content is showed in the figure 1. Regarding nitrate pattern, the values obtained for this parameter remained constant during the manufacture process in the four batches. Significant differences (p<0.05) were found between the batch 1 sausages (with nitrate added) and those the others three batches, however, no differences (p>0.005) were found between the control batch (withoth nitrate added) and the batches 2 and 3 (with vegetable juice powder added).

The nitrite content decreased after fermentation step in the sausages manufactured with nitrite added (batch 1) and then remained constant up to the final (26 days). The nitrite content for the sausages manufactured without nitrite added (control batch, batch 2 and batch 3) showed a slight increase at day 5 due to the nitrate present was reduced to nitrite by the action of the microbial flora with nitrate reductase activity (present in the product or added as starter culture). In all days of processing, the highest values in the nitrite content were found in the sausages manufactured with nitrite added (batch 1). No differences (p>0.05) were found between the others three sausages batches. These fact could be explained due to the amount of vegetable juice powder added initially was not enought. Besides, it could also be due to a low nitrite reuctase activity to the strains added as starter culture. In this way, further studies are neccesary carried out to optimize the use of vegetable juice as sources of nitrates and nitrites in dry fermented meat products.

#### IV. CONCLUSION

In summary, the sausages manufacture process with vegetable juice powder as source of nitrate was similar to the sausages manufacture process without nitrates and nitrites added. For that reason further studies are necessary carried out to optimize the use of vegetable juice as sources of nitrates

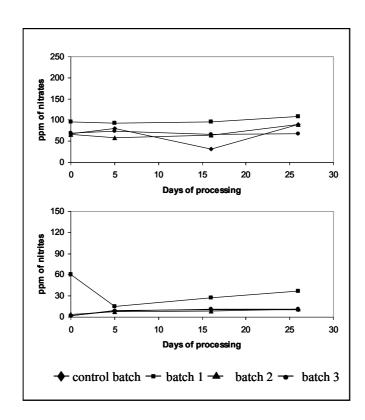
and nitrites in dry fermented meat products.

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**Figure 1.** Evolution of nitrate and nitrite content during the processing of four batches of dry fermented sausage chorizo manufactured: Control batch: without nitrates and nitrites added, batch 1: with nitrates and nitrites added, batch 2: with vegetable juice powder and batch 3: with vegetable juice powder and with starter cultures.

**Table 1.-** Ingredients added to the four differents batches of "chorizo" manufactured.

<del>-</del>	Batch					
Ingredients	Control	1	2	3		
Pork meat (%)	75	75	75	75		
Pork backfat (%)	25	25	25	25		
NaCl (g/Kg)	20	20	20	20		
Paprika(g/Kg)	19	19	19	19		
Garlic (g/Kg)	1.5	1.5	1.5	1.5		
Nitrates (ppm)		150		_		
Nitrites (ppm)	_	100	_	_		
Vegetable juice powder (g/Kg)	_	_	20	20		
Dextrose (g/Kg)	_	10		_		
Polyphosphates (g/Kg)	_	1.0		_		
Ascorbate sodium (g/Kg)	_	0.45		_		
Oregano (g/Kg)	1.0	1.0	1.0	1.0		
Black pepper (g/Kg)	1.0	1.0	1.0	1.0		
Starter culture (g/Kg)	_	_	_	0.6		

**Table 2.** Microbial counts (log cfu/g) obtained during the processing of four batches of "*chorizo*": (Control batch: without nitrates and nitrites added, batch 1: with nitrates and nitrites added, batch 2: with vegetable juice powder and batch 3: with vegetable juice powder and with starter cultures).

		Days of processing			
	Batch	0	5	16	26
Aerobic mesophilic bacteria	Control	<sup>A</sup> 6.67 <sub>a</sub>	<sup>A</sup> 8.80 <sub>b</sub>	<sup>A</sup> 8.65 <sub>b</sub>	<sup>A</sup> 9.15 <sub>c</sub>
	1	<sup>A</sup> 6.61 <sub>a</sub>	<sup>A</sup> 8.82 <sub>b</sub>	<sup>A</sup> 8.70 <sub>b</sub>	<sup>A</sup> 9.26 <sub>b</sub>
	2	<sup>A</sup> 6.72 <sub>a</sub>	<sup>A</sup> 8.66 <sub>b</sub>	<sup>A</sup> 9.07 <sub>c</sub>	<sup>A</sup> 9.09 <sub>c</sub>
	3	<sup>B</sup> 7.40 <sub>a</sub>	<sup>A</sup> 8.70 <sub>b</sub>	<sup>A</sup> 8.93 <sub>b</sub>	<sup>A</sup> 8.95 <sub>b</sub>
Enterobacteria	Control	<sup>A</sup> 3.27 <sub>a</sub>	<sup>B</sup> 5.48 <sub>b</sub>	<sup>A</sup> 6.24 <sub>c</sub>	<sup>c</sup> 6.15 <sub>c</sub>
	1	<sup>c</sup> 3.73 <sub>b</sub>	<sup>B</sup> 5.48 <sub>c</sub>	<sup>B</sup> 6.48 <sub>d</sub>	<sup>A</sup> 3.00 <sub>a</sub>
	2	<sup>c</sup> 3.81 <sub>b</sub>	<sup>A</sup> 2.93 <sub>a</sub>	<sup>A</sup> 6.27 <sub>d</sub>	<sup>B</sup> 5.29 <sub>c</sub>
	3	<sup>B</sup> 3.48 <sub>a</sub>	<sup>B</sup> 5.48 <sub>b</sub>	<sup>B</sup> 6.48 <sub>c</sub>	<sup>c</sup> 6.17 <sub>c</sub>
Lactic acid bacteria	Control	<sup>A</sup> 6.00 <sub>a</sub>	<sup>A</sup> 8.73 <sub>b</sub>	<sup>A</sup> 8.78 <sub>b</sub>	<sup>A</sup> 9.37 <sub>c</sub>
	1	<sup>A</sup> 6.00 <sub>a</sub>	<sup>A</sup> 8.79 <sub>b</sub>	<sup>c</sup> 9.25 <sub>c</sub>	<sup>A</sup> 9.22 <sub>c</sub>
	2	<sup>A</sup> 6.00 <sub>a</sub>	<sup>A</sup> 8.70 <sub>b</sub>	<sup>B</sup> 8.99 <sub>b</sub>	<sup>A</sup> 9.19 <sub>b</sub>
	3	<sup>B</sup> 7.02 <sub>a</sub>	<sup>A</sup> 8.73 <sub>b</sub>	$^{\mathrm{BC}}9.11_{\mathrm{b}}$	<sup>A</sup> 8.87 <sub>b</sub>
Micrococcaceae	Control	<sup>A</sup> 6.39 <sub>a</sub>	<sup>BC</sup> 7.00 <sub>ab</sub>	<sup>A</sup> 6.80 <sub>ab</sub>	<sup>B</sup> 7.47 <sub>b</sub>
	1	<sup>A</sup> 6.35 <sub>a</sub>	AB 6.41 <sub>a</sub>	<sup>A</sup> 6.79 <sub>a</sub>	<sup>A</sup> 6.54 <sub>a</sub>
	2	<sup>A</sup> 6.39 <sub>ab</sub>	<sup>A</sup> 6.07 <sub>a</sub>	<sup>A</sup> 7.17 <sub>b</sub>	<sup>B</sup> 7.16 <sub>b</sub>
	3	<sup>A</sup> 7.20 <sub>a</sub>	<sup>C</sup> 7.23 <sub>a</sub>	<sup>A</sup> 7.35 <sub>a</sub>	<sup>A</sup> 6.78 <sub>a</sub>

<sup>&</sup>lt;sup>a,b,c,d,</sup> Means with different letters in the same row indicate significant differences during the processing (Tukey test: p<0.05).

 $<sup>^{</sup>A,B,C}$  Means with different letters in the same column and for each microbial group indicate significant differences between batches (Tukey test: p<0.05).

**Table 3.** Evolution of weight loss, pH and  $a_w$  during the processing of four batches of "*chorizo*": (Control batch: without nitrates and nitrites added, batch 1: with nitrates and nitrites added, batch 2: with vegetable juice powder and batch 3: with vegetable juice powder and with starter cultures).

		Days of processing				
	Batch		5	16	26	
Weight loss (%)	Control	_	<sup>A</sup> 10.83 <sub>a</sub>	<sup>A</sup> 31.34 <sub>b</sub>	<sup>A</sup> 40.25 <sub>c</sub>	
	1	_	<sup>A</sup> 11.26 <sub>a</sub>	<sup>A</sup> 32.43 <sub>b</sub>	<sup>A</sup> 41.46 <sub>c</sub>	
	2	_	<sup>A</sup> 12.77 <sub>a</sub>	<sup>A</sup> 31.27 <sub>b</sub>	<sup>A</sup> 40.53 <sub>c</sub>	
	3	-	<sup>A</sup> 13.48 <sub>a</sub>	<sup>A</sup> 33.14 <sub>b</sub>	<sup>A</sup> 41.51 <sub>c</sub>	
Hd	Control	<sup>8</sup> 5.68 <sub>c</sub>	<sup>A</sup> 5.20 <sub>a</sub>	<sup>D</sup> 5.55 <sub>b</sub>	<sup>B</sup> 5.85 <sub>d</sub>	
	1	<sup>A</sup> 5.63 <sub>c</sub>	<sup>B</sup> 5.39 <sub>b</sub>	<sup>B</sup> 4.99 <sub>a</sub>	<sup>A</sup> 5.00 <sub>a</sub>	
	2	<sup>c</sup> 5.79 <sub>d</sub>	<sup>A</sup> 5.22 <sub>c</sub>	<sup>A</sup> 4.86 <sub>a</sub>	<sup>A</sup> 4.99 <sub>b</sub>	
	3	<sup>c</sup> 5.81 <sub>d</sub>	<sup>A</sup> 5.26 <sub>c</sub>	<sup>c</sup> 5.14 <sub>b</sub>	<sup>A</sup> 5.05 <sub>a</sub>	
, s	Control	<sup>A</sup> 0.976 <sub>d</sub>	<sup>A</sup> 0.964 <sub>c</sub>	<sup>B</sup> 0.933 <sub>b</sub>	<sup>c</sup> 0.896 <sub>a</sub>	
	1	<sup>A</sup> 0.972 <sub>d</sub>	<sup>A</sup> 0.960 <sub>c</sub>	AB 0.929 <sub>b</sub>	<sup>A</sup> 0.863 <sub>a</sub>	
	2	<sup>A</sup> 0.972 <sub>d</sub>	<sup>A</sup> 0.962 <sub>c</sub>	AB 0.930 <sub>b</sub>	<sup>B</sup> 0.884 <sub>a</sub>	
	3	<sup>A</sup> 0.975 <sub>d</sub>	<sup>A</sup> 0.963 <sub>c</sub>	<sup>A</sup> 0.925 <sub>b</sub>	<sup>B</sup> 0.880 <sub>a</sub>	

a,b,c,d, Means with different letters in the same row indicate significant differences during the processing (Tukey test: p<0.05).

A,B,C, Means with different letters in the same column and for each parameter indicate significant differences between batches (Tukey test: p<0.05).