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Antioxidant activity of rosemary and citrus extract and natural sources of nitrates in clean label "chorizo" (#352)

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Introduction

Natural nitrate sources have been studied in order to find potential substitutes of synthetic nitrates and nitrites used in food products, such as green leaf vegetables rich in nitrates (lettuce, arugula, watercress, celery, and spinach). Addition of synthetic nitrites (E-249 and E-250) to cured meat products act as antioxidant against lipid oxidation, antimicrobial against anaerobic bacteria, and colour preservative. . In addition, in order to adapt our model to new trends of Clean label meat product elaboration, synthetic additives have been substituted by natural nitrate sources to create a Spanish "chorizo" free of synthetic additives. The use of natural sources of nitrates from green leaf vegetables could prevent the abuse of synthetic nitrates in the development of Clean Label food products.

Methods

Plant, spices and vegetables extracts. Natural antioxidant extracts were obtained from citrics (Citrus sinensis L.) (C), with 55.11 % hesperidin and rosemary (Rosmarinus officinalis L.) (R), with 14.59 % carnosic acid, 5.84 % carnosol and 0.60 % 12-O-methylcarnosic acid. Both of them were supplied by Nutrafur-Frutarom, S. L. Paprika, garlic, and oregano powder, as well as fresh vegetables lettuce, arugula, spinach, celery, and watercress were bought in a local supermarket (Hipercor, S. A., El Tiro, Murcia, Spain).

Antioxidant activity. The antioxidant activity related with the chelating power to different free radicals (%) was firstly measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), DPPH, FRAP and ORAC.

Chorizo elaboration. The meat mixed with the starter cultures, additives, spices, and natural extracts according to Table 1.

Protein oxidation. Protein oxidation in chorizo was related with the thiol concentration.

Statistic analysis.Data were analyzed with the statistical package SPSS 15.0 (Statistical Package for the Social Science for Window (IBM, Armonk, New York, USA).

Results

Taking into account results presented data in Table 2, it can be reported that Acerola and R showed the highest chelating activity against DPPH and ABTS radical cations. Regarding the scavenging capacity against DPPH radical cation, it can be appreciated as these extracts are followed by the rest of traditional ingredients and leafy green vegetables comprended in a small range among 51.5 until 41.3 %: Garlic powder, Lettuce, Arugula, Celery, Paprika, Watercress, Spinach and Oregano, in this order. Lastly C with a 8.45 % scavenging against the lipohilic radical

Moreover, the scavenging activity against the hydrophilic radical, ABTS, is generally lower than against DPPH. For this reason, the scavenging activity against ABTS followed the next hierarchy: Watercress, Garlic, Arugula, Paprika, Spinach, Oregano, C, Lettuce, and Celery with values among 33.4 until 12 %, after R, and Acerola that presented values of 85.7, 70.2, and 46.5 %, respectively.

In addition, it can be observed a similar behaviour regarding to the efficiency of each extract to reduce Fe+3 to Fe+2 comparing to the hydrophilic antioxidant capacity measured by their oxygen radical absorbance. In this case, applying FRAP method, natural extracts rich in phenolic compounds are the first of the list: R, from rosemary, Oregano, C, from citrics, Watercress and Paprika followed by Arugula, Lettuce, Spinach, Acerola, Garlic and Celery, the last one with 804.7 µM TE 100 g-1, 50 % less than Garlic with 1915.7, or 95 % less than R with 17790 µM TE 100 g-1. Otherwise, obtained results from the analysis of the oxygen radical absorbance showed the next hierarchy, with similar values to published by USDA in 2010: R > Oregano > Paprika > C > Arugula ≥ Garlic ≥ Lettuce ≥ Acerola ≥ Spinach ≥ Watercress > Celery.

Results of protein oxidation related with thiol group loss for 150 days of refrigerated storage are shown in Table 5. The concentration of protein thiols in the Control "chorizo" sample was found to be 55.2 ± 2.2 mmol/mg protein, which is comparable to previous results reported by Jongberg, Tørngren & Skibsted (2018) in brine-injected pork loins. Then, a gradually decreasement in the concentration of thiol groups was observed in all the Spanish "chorizo" samples. This In addition, in this investigation, R produced an increasement in thiol loss, which might be produced by reactions between phenolic compounds from rosemary and free thiol groups that may form thiol-quinone adducts.

Conclusion

Natural extracts with the highest antioxidant capacity were Rosemary and Citrus. The incorporation of these extracts as preservatives in Spanish "chorizo" presented excellent antioxidant capacities to avoid the protein oxidation and it could be an excellent strategy in order to produce Clean Label cured meat products. Nitrate natural sources obtained from leafy green vegetables presented synergism, specially with the combination formulated with Lettuce, Arugula, and Watercress combined with Citric extract as antioxidant, however, more studies are required to do this conclusions.

Notes

Table 2. Antioxidant activity of natural extracts by measuring their ABTS, and DPPH radical scavenging activity, together to their $ORAC_{HP}$, and FRAP (μM TE g^{-1}) (M ± SD).

	Chelating activity percent (%)		Antioxidant activity ($\mu M\ TE\ g^{-1} \pm SD$)		
Samples	**ABTS**	**DPPH**	**$ORAC_{HP}$**	**FRAP**	**$ORAC_{HP}$ (USDA, 2010)**
C	15.4 ± 0.2^{h}	8.45 ± 0.3^{k}	4828.5 ± 19.9^{d}	6004.7 ± 29.6^{c}	*Nd*
Acerola	46.5 ± 0.3^{c}	78.3 ± 0.5^{b}	1680.7 ± 19.3^{g}	1925.7 ± 28.7^{f}	*Nd*
R	70.2 ± 0.1^{b}	76.7 ± 1.7^{c}	19909.0 ± 59.8^{a}	17790 ± 53.3^{a}	*112200***
Paprika	21.1 ± 1.6^{f}	48.7 ± 0.2^{ef}	5746.0 ± 21.7^{c}	2491.3 ± 17.1^{e}	*13750*
Garlic	25.4 ± 0.8^{e}	51.5 ± 0.3^{d}	1919.3 ± 23.4^{g}	1915.7 ± 52.5^{f}	*450*
Oregano	15.6 ± 0.5^{h}	41.3 ± 0.2^{j}	11436.7 ± 27.5^{b}	9355.3 ± 46.4^{b}	*13970*
Lettuce	14.6 ± 1.1^{i}	49.9 ± 0.1^{e}	1723.3 ± 35.1^{g}	1998 ± 18.9^{f}	*1321**
Arugula	25.9 ± 3.1^{e}	49.2 ± 1.2^{e}	2881.3 ± 28.4^{f}	2071 ± 16.3^{ef}	*1904**
Spinach	20.1 ± 0.1^{g}	43.6 ± 3.6^{i}	1491.3 ± 22.1^{gh}	1995.3 ± 9.6^{f}	*1513**
Celery	12.0 ± 0.5^{j}	48.7 ± 0.4^{ef}	993.7 ± 18.5^{i}	804.7 ± 33.6^{g}	*512**
Watercress	33.4 ± 2.6^{d}	46.5 ± 0.1^{h}	$1200.7 \pm 15.0h$	2510.3 ± 39.4^{e}	*Nd*

Superscript letters indicate significant differences ($p < 0.05$) between natural extracts. M ± SD: Mean ± Standard deviation; TE: Trolox Equivalents. *Theese data correspond to fresh vegetables. **This data corresponds to dried rosemary. Nd: no data found.

Table 2

Table 3. Protein oxidation (thiol groups) in Spanish "chorizo".

	Days of ripening		Days of vacuum-packed storage						
Sample	**0**	**2**	**10**	**25**	**50**	**75**	**100**	**125**	**150**
Protein oxidation: Thiol groups									
Control	55.2 ± 2.2^{v}	46.8 ± 1.1^{w}	37.4 ± 2.9^{ax}	18.6 ± 1.2^{ay}	9.8 ± 0.5^{bz}	6.4 ± 1.7^{bz}	12.3 ± 1.1^{ayz}	12.4 ± 1.1^{ayz}	9.6 ± 0.9^{bz}
R_{LAW}	58.1 ± 1.9^{v}	45.4 ± 1.4^{w}	29.4 ± 2.1^{bx}	11.8 ± 0.6^{by}	11.1 ± 0.8^{by}	9.4 ± 0.5^{byz}	11.0 ± 0.7^{ay}	7.7 ± 0.3^{byz}	9.8 ± 1.0^{byz}
R_{SCe}	51.4 ± 4.1^{v}	42.1 ± 2.3^{w}	25.5 ± 2.6^{bx}	19.5 ± 1.6^{ay}	14.2 ± 1.1^{abyz}	6.8 ± 0.8^{bz}	3.46 ± 0.1^{cz}	5.6 ± 0.3^{bcz}	2.9 ± 0.5^{cz}
C_{LAW}	55.9 ± 3.2^{v}	43.1 ± 2.8^{w}	25.7 ± 1.6^{bx}	18.0 ± 1.1^{ay}	17.1 ± 1.0^{ay}	9.0 ± 0.7^{byz}	14.1 ± 1.2^{ay}	8.4 ± 0.9^{byz}	12.9 ± 0.5^{ayz}
C_{SCe}	55.3 ± 4.0^{v}	45.2 ± 3.5^{w}	26.2 ± 2.0^{bx}	20.2 ± 1.5^{axy}	19.2 ± 1.1^{ay}	14.4 ± 1.5^{ay}	9.4 ± 0.8^{byz}	9.3 ± 0.8^{byz}	3.6 ± 0.3^{cz}

R_{LAW}: 500 ppm Rosemary extract + 250 ppm Acerola + 3000 ppm Lettuce, Arugula, and Watercress; R_{SCe}: 500 ppm Rosemary extract + 250 ppm Acerola + 3000 ppm Spinach and Celery; C_{LAW}: 500 ppm Citric extract + 250 ppm Acerola + 3000 ppm Lettuce, Arugula, and Watercress; C_{SCe}: 500 ppm Citric extract + 250 ppm Acerola + 3000 ppm Spinach and Celery. Different subscript letters indicate significant differences ($p<0.05$) between samples (a, b, c) and day of analysis (v, w, x, y, z)

Table 3

Table 1. Formulation of Spanish "chorizo" samples.

	Samples				
Ingredients	**Control**	**C_{LAW}**	**C_{SCe}**	**R_{LAW}**	**R_{SCe}**
Pork meat (g)	*875*	*875*	*875*	*875*	*875*
Pork fat (g)	*1350*	*1350*	*1350*	*1350*	*1350*
Water (ml)	*75*	*75*	*75*	*75*	*75*
Commercial mix® (g/kg)	*65*				
Paprika (g/kg)		*30*	*30*	*30*	*30*
Oregano (g/kg)		*3*	*3*	*3*	*3*
Garlic (g/kg)		*3*	*3*	*3*	*3*
Dextrose (g/kg)		*3*	*3*	*3*	*3*
Salt (g/kg)		*5*	*5*	*5*	*5*
Meat protein (g/kg)		*23*	*23*	*23*	*23*
Ferment (ml)	*20*	*20*	*20*	*20*	*20*
Natural extracts (ppm):					
C		*200*	*200*		
R				*200*	*200*
Acerola		*100*	*100*	*100*	*100*
LAW		*3000+1500+1500*		*3000+1500+1500*	
SCe			*3000+3000*		*3000+3000*

Commercial mix®: composed by: Spices, salt, dextrose, lactose, milk protein, emulsifiers (triphosphates E-451, diphosphate E-450), flavour enhancer (monosodium glutamate E-621), preservative (sodium nitrate E-251), antioxidant (sodium ascorbate E-301), and colouring (carminic acid E-120). C: Citric; R: Rosemary; LAW: Lettuce + Arugula + Watercress; SCe: Spinach + Celery.

Table 1

Notes