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ORIGINAL PAPER

Orange fibre as potential functional ingredient for dry-cured sausages

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Abstract Dry-cured sausages with orange fibre (juice industry by-products) at five concentrations (0, 5, 10, 15 and 20 g/kg) were prepared and studied. Chemical (residual nitrite level and lipid oxidation) and physico-chemical parameters (colour and pH) were determined during dry-curing stage (4 weeks). Polyphenol composition (extracted with methanol and separated and quantified by HPLC) of each formulation and its evolution during dry-curing were also determined. TBARS values increased in all samples during drying, with lower increase in treatment samples than in control samples. The incorporation of orange fibre into sausages produced a significant decrease in residual nitrite level. The high reactivity of nitrites could allow its reaction with active biocompounds (polyphenols) present in orange fibre. Chromatographic (HPLC) analysis of orange fibre detected numerous peaks, most of them matched with phenolic compounds. The highest peak of this chromatogram was identified as hesperidin. So, hesperidin was selected as the most adequate compound to monitor polyphenols changes in sausages added with orange fibre during processing.

Keywords Orange fibre · Fermented dry-cured sausages · Dietary polyphenols · Antioxidant properties

Introduction

Dry-cured sausages manufacture is a major industry in Spain. They are manufactured from a mixture of chopped pork meat, lard, salt, additives (nitrate, nitrite, and antioxidants), starter cultures (optional) and spices. Once sausage batter is mixed and cased it is subjected to fermentation and ripening [1–3].

An excessive intake of meat products, particularly dry-fermented sausages, is not recommended from a health point of view, at least for some population groups, due to their high level of sodium and animal fat [4]. Concerning fat supply, it is well known that the lean meat and fat used in the elaboration of these products contains higher proportion of saturated fatty acids than polyunsaturated fatty acids. Health organizations all over the world have promoted the choice of a diet low in saturated fat and moderated in total fat, as a means of preventing cardiovascular disease [4, 5].

Increased concerns about the potential health risk associated with the consumption of high-fat foods have led the food industry to develop new formulations or modify traditional products to make them healthier. Recent research has focused on the use of different types of fibres (inulin, cereal and fruit fibres) as partial substitutes of pork backfat in dry-fermented sausages [6, 7].

The citrus fruit (oranges, lemon, grapefruits) industry is important in tropical and subtropical zones and especially in Spain, which is one of the major producers and exporters. Their processing produces a considerable amount of by-products, which are a problem since the plant material is usually prone to microbial spoilage and commonly they are only used as animal feed or fertilizer [8, 9]. Isolation of functional compounds from citrus by-products (fibre and polyphenols) can be of interest to the food industry as they can retard oxidative changes in food and thereby improve the quality and nutritional value of food. Today, there is strong evidence supporting a role for polyphenols in prevention of age-related diseases [10, 11]. In this respect, it is noteworthy that the nutrition community has recognized the importance of dietary polyphenols as health-promoting agents. Recent

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studies reported the application of different plants rich in phenolics as potential food ingredients [12]. Salminen et al. [13] applied by-products of deoiling processes (rapeseed, camelina and soy meal) rich in phenolics, to cooked pork meat patties and they concluded that all of them were effective in protecting cooked meat product from lipid and protein oxidation.

Different types of citrus fibre have been successfully used in the processing of fresh meat products [14, 15], cooked meat products [16, 17] and non-fermented dry-cured meat products [18, 19]. Until the moment, no reports have been published on the effect of the addition of citrus fibre on the processing of fermented dry-cured sausages.

This study describes research carried out to investigate the effect of the addition of orange fibre (juice industry by-products) on the oxidation of a Spanish dry-fermented sausage in relation to the polyphenols present in the orange fibre.

Materials and methods

Orange fibre

Orange fibre was obtained directly from by-products of a local orange juice industry following the optimized procedure described by Fernández-Ginés et al. [16].

The powder obtained was vacuum packed until used.

Sausage manufacture

Three independent replicates of each sausage were made. Sausages were manufactured in the Food Pilot Plant from Miguel Hernández University according to a traditional formulation (meat amounts add up to 1 kg and quantities of others ingredients are related to meat): 500 g lean pork meat, 500 g pork backfat, 50 g/kg water, 28 g/kg sodium chloride, 10 g/kg lactose, 0.5 g/kg sodium ascorbate, 0.25 g/kg sodium nitrite and 2 g/kg black pepper. This original mixture was split into batches to which orange fibre was added in different amounts: 0, 5, 10, 15 and 20 g/kg.

The meat and fat cuts were minced through a 6-mm plate (Cato, Sabadell, Spain), mixed with other ingredients and stuffed in artificial casings of 40–43 mm diameter. Fermentation, drying and curing were carried out at $T = 15 \pm 1\,^{\circ}\mathrm{C}$ and 75% relative humidity for 4 weeks.

Samples were taken from the original sausages (day 0) and each week during the 4 weeks of dry-curing time.

Chemical analysis

Residual nitrite level (mg $NaNO_2$/kg sample) was determined by following standards ISO/DIS 2918 [20].

Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid (TBA) method of Buege and Aust [21]. Thiobarbituric acid reactive substances (TBARS) values were calculated from a standard curve of malonaldehyde (MA) and expressed as mg MA/kg sample.

Physico-chemical analysis

The CIE LAB colour space was studied following the procedure of Cassens et al. [22]. The following colour coordinates were determined: lightness (L^*), redness (a^*, +/− red-green) and yellowness (b^*, +/− yellow-blue). Colour determinations were made using a reflectance spectrophotometer Minolta CM-2600 (Minolta Camera Co., Osaka, Japan) with illuminant D_{65}, 10° observer, SCI mode, 11 mm aperture of the instrument for illumination and 8 mm for measurement, and spectrally pure glass (CR-A51, Minolta Co., Osaka, Japan) was put between the samples and the equipment.

The pH was measured with a combination electrode pH meter (Model 507, Crison, Barcelona, Spain) on the suspension obtained by blending a 15 g sample with 150 ml deionized water for 2 min.

Polyphenolic compounds determination

Extraction of polyphenols

Samples (1 g orange fibre or 5 g sausage) were weighed into a tube test and 15 ml of methanol were added. The mixture was vigorously shaken for 2 min and left for 2 h in an ultrasonic water bath without temperature control. Then, the mixture was filtered through an Albet nylon filter (Albet, Barcelona, Spain) of 45 μm diameter. The mixture was transferred to a round-bottomed flask and the methanol was evaporated to dryness using a rotary evaporator under reduced pressure (< 100 mbar) at $T = 40\,^{\circ}\mathrm{C}$. Five millilitres of dimethyl sulfoxide (DMSO) were added to the residue, and the mixture was well shaken by hand for 2 min. The solution was filtered through a 0.45 μm membrane filter before HPLC analysis.

HPLC analysis

The HPLC analysis was performed using a Hewlett Packard HP-1100 instrument (Woldbronn, Germany) equipped with a photodiode array detector and a C-18 column (Lichrospher, 250-4, Waters) at $T = 30\,^{\circ}\mathrm{C}$. Phenolic compounds were analysed in standard and sample solutions using gradient elution at 1 ml/min with gradient program (0–20 min 95–75% A, 20–40 min 75–50% A, 40–50 min 50–20% A, 50–60 min 20% A) with 2.5% acetic acid in water as solvent A and methanol as solvent B [23].

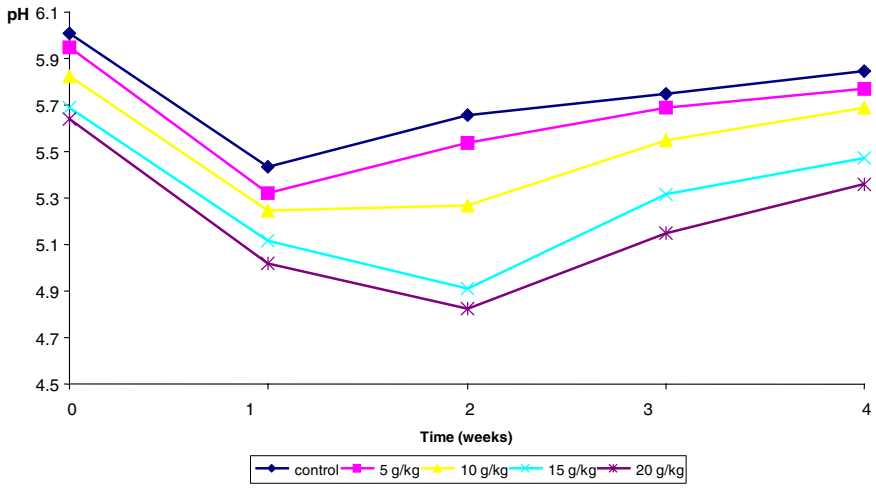

Fig. 1 Development of pH during dry-curing of sausages added with different concentrations of orange fibre

Phenolic compounds (eriocitrin, neoeriocitrin, rutin, luteolin-7-*O*-glucoside, hesperidin, diosmin, poncirin, hesperetin, neodiosmin) were identified by comparing retention times with photodiode array spectra, in the range 220–500 nm for standards (Sigma, Steinheim, Germany) and samples; and quantified by computing the peak areas.

Statistical analysis

Each variable was determined in triplicate. Analysis of variance (ANOVA) with two factors (fibre concentration and dry-curing time) was used to determine significant differences ($P < 0.05$) between treatments. To assess differences between the levels of the main factor, comparisons between means (Tukey test) were used [24]. The statistical analyses were done using Statistica for Windows Release 5.0A [25].

Results

pH

pH levels showed differences ($P < 0.05$) for orange fibre concentration and processing time. The pH in control sausages showed a decrease ($P < 0.05$) during the first week and then an increase back to similar values by the end of the drying time (Fig. 1). This pH evolution is similar to that reported for other dry-cured meat products [1, 26, 27]. The decrease in pH during the first week may is due to microbial activity, since the microorganisms metabolize the sugars present in the meat batter into lactic acid [28]. Sausages added with 5 g/kg orange fibre showed a similar ($P > 0.05$) pH evolution than control samples. In contrast, the addition of more than 5 g/kg of orange fibre decreased ($P < 0.05$) pH, which may be due to the presence of organic acids and other acid compounds in orange fibre (orange fibre pH $= 3.28 \pm 0.09$) [29, 30]. This decrease was higher ($P < 0.05$) when more fibre was added. A decrease in pH has also been reported by our research group in cooked products and non-fermented dry-cured sausages added with lemon albedo [17–19]. As can be seen in Fig. 1, sausages added with 15 and 20 g/kg orange fibre showed a decrease in pH not only during the first week but also during the second week.

Lipid oxidation (TBARS)

Lipid oxidation was evaluated through the determination of TBARS values (Fig. 2). TBARS showed differences ($P < 0.05$) for orange fibre concentration and processing time. TBARS values increased in all samples during drying, although the increase in sausages formulated with orange fibre was lower than in control samples. It is important to observe that, for all samples, this increase in TBARS during drying time was lower during the last week. It could be due to malonaldehyde degradation in other volatile compounds, which could not be detected by TBARS assay [18, 19, 31, 32]. The increase in TBARS values during drying has been reported in other dry-cured sausages [33, 34], and it has been attributed to lipid oxidation reactions also involved in the characteristic flavour development of this type of fermented sausages. The processing conditions of dry-cured sausages including mincing, mixing with salt and drying for long time, appear to be significantly prooxidant [35]. When more orange fibre was added, lower TBARS values were reached at the end of the drying stage. This behaviour could indicate that orange fibre has a protective effect from the oxidation process.

Colour parameters

Lightness, redness and yellowness showed differences ($P < 0.05$) for orange fibre concentration and dry-curing

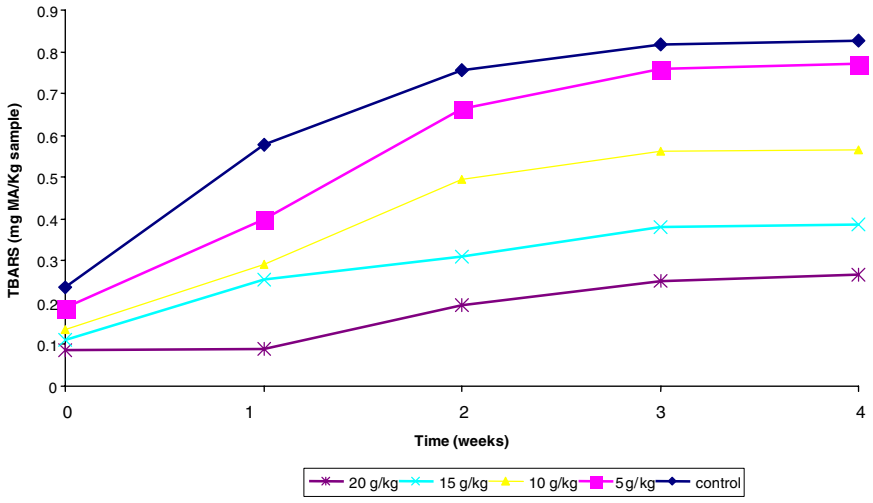

Fig. 2 Development of TBARS during dry-curing of sausages added with different concentrations of orange fibre

Table 1 Development of colour parameters (lightness (L^*) redness (a^*) and yellowness (b^*)) during dry-curing of sausages added with different concentrations of orange fibre

		Orange fibre				
	Time (weeks)	Control	5 g/kg	10 g/kg	15 g/kg	20 g/kg
L^*	0	48.39 ± 0.44	48.42 ± 0.56	48.78 ± 0.18	48.75 ± 0.32	48.56 ± 0.19
	1	45.93 ± 0.6b	47.08 ± 0.39	47.27 ± 0.76	46.82 ± 0.28	46.89 ± 0.39
	2	45.26 ± 0.37	46.32 ± 0.78	46.00 ± 0.12	46.40 ± 0.31	46.56 ± 0.29
	3	44.74 ± 0.39	45.74 ± 0.58	45.53 ± 0.61	45.48 ± 0.42	45.89 ± 0.39
	4	44.74 ± 0.42	45.58 ± 0.38	45.36 ± 0.56	45.45 ± 0.31	45.77 ± 0.23
a^*	0	5.99 ± 0.12	6.02 ± 0.07	6.64 ± 0.13	6.74 ± 0.11	6.86 ± 0.12
	1	6.69 ± 0.09	6.64 ± 0.12	7.38 ± 0.07	7.55 ± 0.05	7.57 ± 0.05
	2	5.94 ± 0.09	5.98 ± 0.08	6.90 ± 0.10	7.02 ± 0.07	7.20 ± 0.09
	3	5.29 ± 0.11	5.31 ± 0.10	6.40 ± 0.08	6.60 ± 0.11	6.64 ± 0.06
	4	4.92 ± 0.13	4.98 ± 0.14	6.17 ± 0.12	6.48 ± 0.05	6.43 ± 0.07
b^*	0	30.70 ± 1.12	29.41 ± 0.99	29.51 ± 1.35	28.95 ± 1.36	26.08 ± 1.36
	1	25.95 ± 1.07	27.04 ± 0.93	27.83 ± 1.32	33.60 ± 1.20	28.18 ± 1.56
	2	21.88 ± 0.99	17.48 ± 1.16	22.33 ± 1.36	18.98 ± 1.06	20.07 ± 1.23
	3	25.66 ± 1.04	19.74 ± 1.45	25.61 ± 1.03	25.30 ± 1.22	25.60 ± 1.29
	4	25.06 ± 1.88	26.84 ± 1.36	20.97 ± 1.12	17.81 ± 1.35	19.92 ± 1.16

time (Table 1). Lightness decreased during dry-curing time in all samples, although this decrease was higher ($P < 0.05$) in control samples than in samples added with orange fibre. Samples added with orange fibre (at any concentration) showed higher ($P < 0.05$) L^* values than control samples during all drying times. The decrease in L^* during dry-curing time could be attributed to moisture losses, as expected in dry-cured sausages [1, 27]. The effect of orange fibre on lightness evolution could be related to pH and water distribution in the meat matrix. Some authors have reported that lightness in meat products depends on the effect on pH on meat protein structure [36, 37]. When the pH is lower than the isoelectric point of the meat proteins those proteins are denatured and their structure is altered, thus modifying their light reflection and refraction properties [38].

Redness showed differences ($P < 0.05$) when more than 5 g/kg of orange fibre was added. Samples added with more than 5 g/kg of orange fibre showed higher ($P < 0.05$) a^* values than control and 5 g/kg orange fibre added ones. In all samples, redness increased ($P < 0.05$) during the first week and afterwards decreased ($P < 0.05$). This increase in a^* values during the first week could be attributed to nitrosomyoglobin formation [39] and also to moisture loss, which provokes an increase in heme pigments concentration [1]. The decrease in a^* values observed after the first week of dry-curing time could be related to lipid oxidation. This behaviour has been also observed in other dry-cured meat products [1, 27]. The antioxidant effect attributed to orange fibre when it is added in amounts 10 g/kg or higher (as has been discussed above in lipid oxidation section), could also explain the lower decrease in a^* values for sausages added with more than 5 g/kg of orange fibre.

There were no differences for b^* values ($P > 0.05$) between control and samples added with 5 g/kg orange fibre. Yellowness showed differences ($P < 0.05$) when more than 5 g/kg of orange fibre was added. These sam-

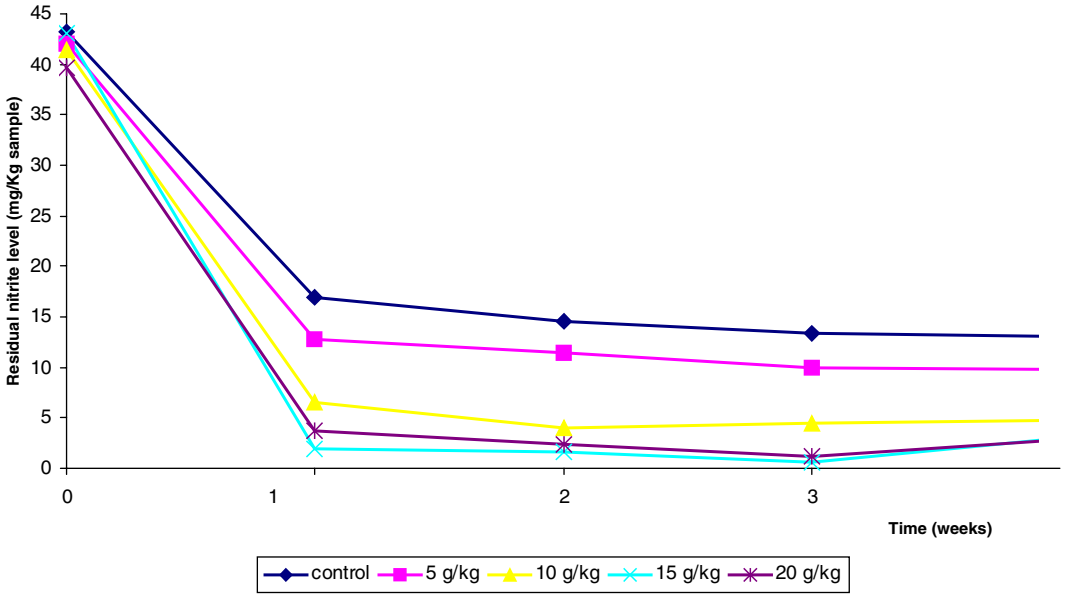

Fig. 3 Development of residual nitrite level during dry-curing of sausages added with different concentrations of orange fibre

ples showed higher b^* values than control and 5 g/kg ones. These differences can be appreciated even at day 0, so the presence of yellow compounds (carotenes) in orange fibre could have contributed to this value. Yellowness (b^*) decreased during dry-curing period, this decrease being higher (aprox. 5 units) in the first week than in the rest of the period (aprox. 1 unit). The decrease in yellowness during dry-curing stage of dry-cured sausages has been attributed to the modifications in myoglobin stages [40].

Residual nitrite level

Nitrite added in the formulation is reduced to nitric oxide that reacts with myoglobin to form nitric oxid myoglobin, which gives the characteristics cured meat red colour [41]. The values of residual nitrite level correspond to nitrite that has not reacted with myoglobin and it is available. Residual nitrite level showed differences ($P < 0.05$) for orange fibre concentration and processing time. The incorporation of orange fibre to sausages produced a significant decrease ($P < 0.05$) in residual nitrite level (Fig. 3). Similar results in nitrite behaviour have been observed in cooked meat products added with citrus fibre [16, 17, 30] and in non-fermented sausages added with lemon albedo [18, 19, 30], but no reports about its have been found in fermented sausages. The high reactivity of nitrites could have allowed its reaction with active biocompounds (polyphenols) present in the orange fibre. Krishnaswamy [42] and Garrote et al. [43] reported that some polyphenolic compounds showed a high protection against nitrite ion, and so prevented nitrosamines and nitrosamides formation in foods.

Polyphenols

Chromatographic analysis of orange fibre detected numerous peaks, all of them matched with phenolic compounds. Eriocitrin, neoeriocitrin, rutin, luteolin-7-*O*-glucoside, hesperidin, diosmin, poncirin, hesperetin and neodiosmin were identified. The highest peak corresponded to hesperidin. Some authors have reported that the principal flavonoid in orange is hesperidin [44]. So, hesperidin was selected as the most adequate compound to follow polyphenols evolution in sausages added with orange fibre during processing. In both, orange fibre enriched sausages and orange fibre itself, hesperidin was the highest peak identified and quantified in the chromatograms.

Hesperidin in sausage samples showed differences ($P < 0.05$) for orange fibre concentration but not ($P > 0.05$) for processing time. There were differences between all fibre concentrations. Sausages with 20 g/kg orange fibre showed the highest hesperidin concentration (0.062 ± 0.009 mg/g), followed by samples with 15, 10 and 5 g/kg (0.046 ± 0.006, 0.034 ± 0.004 and 0.018 ± 0.002 mg/g, respectively) and it was not detected in control sample. Hesperidin has a low water and fat solubility [45]. This property could explain its low evolution during processing time.

Conclusions

The use of orange fibre as ingredient for dry-cured sausages has a protective effect from oxidation and due to the decrease in residual nitrite level could prevent nitrosamines and nitrosamides formation. The most important phenolic

compound in the orange fibre is hesperidin, which has also been detected in sausages added with this fibre. Results suggest that orange fibre is a potential functional ingredient and further research is needed about their potential application in other processed meats.

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References

1. Pérez-Fernández JA, Sayas-Barberá ME, Fernández-López J, Aranda-Catalá V (1999) Food Res Int 32:599–607
2. Fernández-Fernández E, Vázquez-Odériz MA, Romero-Rodríguez MA (2002) Meat Sci 62:67–71
3. Ruiz Pérez-Cacho MP, Galán-Soldevilla H, León-Crespo F, Molina-Recio G (2005) Meat Sci 71:620–633
4. Muguerza E, Gimeno O, Ansorena D, Astiasarán I (2004) Trends Food Sci Technol 15:452–457
5. USDA. US Department of Agriculture–US Department of Health and Human Services (2000) Dietary guidelines for Americans Homes and garden bulletin no. 232. Government University Press, Washington, DC
6. Mendoza E, García ML, Casas C, Selgas MD (2001) Meat Sci 57:387–393
7. García ML, Domínguez R, Gálvez MD, Casas C, Selgas MD (2002) Meat Sci 60: 227–236
8. Famyima O, Ough C (1982) Am J Enol Vitic 33: 44–46
9. Nikolic JA, Cuperlovic M, Milijic C, Djordjevic D, Krsmanovic J (1986) Acta Veter 36:13–22
10. Lau F, Shukitt-Hale B, Joseph J (2006) Neurobiol Aging 26:123–128
11. Bastiaretto S, Yao Zhi-Xing, Papadopoulos V, Quirion R (2006) Eur J Neurosci 23:55–64
12. Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y (2005) Food Rev Int 21:139–166
13. Salminen H, Estévez M, Kivikari R, Heinonen M (2006) Eur Food Res Technol 223:461–468
14. Aleson-Carbonell L, Fernández-López J, Pérez-Alvarez JA, Kuri V (2005) Innov Food Sci Emerg Technol 6:247-255
15. Aleson-Carbonell L, Fernández-López J, Pérez-Alvarez JA, Kuri V (2005) Food Sci Technol Int 11:89–97
16. Fernández-Ginés JM, Fernández-López J, Sayas-Barberá ME, Sendra E, Pérez-Alvarez JA (2003) J Food Sci 68:710–715
17. Fernández-Ginés JM, Fernández-López J, Sayas-Barberá ME, Sendra E, Pérez-Alvarez JA (2004) Meat Sci 67:7–13
18. Aleson-Carbonell L, Fernández-López J, Sayas-Barberá ME, Sendra E, Pérez-Alvarez JA (2003) J Food Sci 68:1826–1830
19. Aleson-Carbonell L, Fernández-López J, Sendra E, Sayas-Barberá ME, Pérez-Alvarez JA (2004) J Sci Food Agric 84:2077–2084
20. ISO (1975) International Standard 2918. Meat and meat products: determination of nitrite content. ISO 2918:1975 (E), International Organization for Standardization, Geneva
21. Buege JA, Aust SD (1978) Methods Enzymol 52:302–310
22. Cassens RG, Demeyer D, Eikelenboom G, Honikel KO, Johansson G, Nielsen T, Renerre M, Richardson Y, Sakata R (1995) In: Proceedings of the 41th international congress of meat science and technology. American Meat Science Association, San Antonio, TX
23. Benavente-Garcia O, Castillo J, Lorente J, Ortuño A, Del Rio JA (1999) Food Chem 68:457–462
24. Afifi AA, Azen SP (1979) Statistical analysis. A computer oriented approach. Academic, London
25. Statistica (1989) Statistica for Windows Release 5.0A. Statsoft Inc, USA
26. Roncalés P (1994) In: Flores J, Bernell S, Toldrá F (eds) Tendencias en el curado de productos cárnicos Expocarne. Valencia, pp 3–23
27. Pagán-Moreno MJ, Gago-Gago MA, Pérez-Alvarez JA, Sayas-Barberá ME, Rosmini MR, Perlo F, Aranda-Catalá V (1998) Fleischw 78:987–989
28. Flores J, Alvarruiz A (1985) Revista de Agroquimica y Tecnología de Alimentos 25:233–239
29. Larrauri JA (1999) Trends Food Sci Technol 10:3–8
30. Fernández-López J, Fernández-Ginés JM, Aleson-Carbonell L, Sayas-Barberá E, Sendra E, Pérez-Alvarez JA (2004) Trends Food Sci Technol 15:176–185
31. Chasco J, Beriain MJ, Bello J (1993) Meat Sci 34:191–204
32. Lorenzo JM, Michinel M, López M, Carballo J (2000) J Food Compos Anal 13:809–817
33. Zanardi E, Novelli E, Ghiretti GP, Chizzolini R (2000) Meat Sci 55:169–175
34. Zanardi E, Dorigoni V, Badiani A, Chizzolini R (2002) Meat Sci 61:7–14
35. Chizzolini R, Novelli E, Zanardi E (1998) In: Proceedings of the 44th international congress of meat science and technology. IRTA, Barcelona, Spain
36. Hunt MC, Acton JC, Benedict RC, Calkins CR, Cornforth DP, Jeremiah LE, Olson DP, Salm CP, Savell JW, Shivas SD (1991) Guidelines for meat color evaluation. American Meat Science Association and National Live Stock and Meat Board, Chicago, USA
37. Fernández-López J, Pérez-Alvarez JA, Sayas-Barberá E, López-Santoveña F (2002) J Food Sci 67:2410–2414
38. Swatland HJ (1995) On line evaluation of meat. Technomic Publishing, Basel, Switzerland
39. Bruggen KA (1994) Dry-sausages technology I. Meat Science School. Viskase Corporation, Chicago
40. Fernández-López, J, Pérez-Alvarez JA, Sayas-Barberá E, Aranda-Catalá V (2000) J Muscle Foods 11:57–68
41. Pegg BR, Shahidi F (1996) Food Chem 56:105–110
42. Krishnaswamy K (2001) Nonnutrients and cancer prevention. ICRM bulletin 31. Available at: http://www.icmr.nic.in
43. Garrote G, Cruz JM, Moure A, Domínguez H, Parajó JC (2004) Trends Food Sci Technol 15:191–200
44. Manthey JA, Grohmann K (1996) J Agric Food Chem 44:811–814
45. Merck (1996) The Merck index. Merck & Co, Inc, New York, USA