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S-Sulfonate Contents in Raw and Cooked Meat Products

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S-Sulfonates ($R-S-SO_3^-$) are compounds formed by the reaction between the sulfites added to foodstuffs and the disulfide bonds of cystine, peptides, and proteins. The content of *S*-sulfonates has been determined in raw sausages and burgers ($n = 62$). The range of variation in the contents of the determined *S*-sulfonates is very wide and varies between 47 and 267 μg of SO_2/g. The degree of formation of *S*-sulfonates with regard to the determined sulfite (total SO_2 + *S*-sulfonates) is similar in all of the samples and does not seem to be conditioned by the meat compound (chicken or beef) or by the process of elaboration or type of product (burgers or sausages). In grilled burgers ($n = 20$) significant losses are produced in the levels of the additive in any of its forms. The value for the *S*-sulfonates is 31 ± 9.8%, 29 ± 6.6% corresponding to the free sulfite and a very similar percentage to the total sulfite (free + reversibly bound) 28 ± 6.7%. It is possible that during the cooking process cleavages of some bound compounds occur, releasing SO_2 and reacting to form new adducts.

KEYWORDS: ***S*-Sulfonates; sulfites; raw and cooked burgers; raw sausages**

INTRODUCTION

The principal function of the sulfites in raw meat products is attributed to their antimicrobial activity (*1*−*3*), which allows the prolongation of the shelf life of these products stored in refrigeration. Moreover, these additives contribute to the maintenance of the color due to their antioxidant activity (*4*, *5*).

The European normative authorizes the addition of sulfites to burger meat with a minimum content of cereals and/or vegetables of 4%, breakfast sausages, and two types of traditional Spanish raw sausages, at maximum residual levels of 450 mg of total SO_2/kg (*6*). However, in other countries sulfur dioxide is prohibited in these kinds of foods (*7*). Thus, in the United States, sulfiting agents are not permitted in meat or in foods recognized as a source of vitamin B_1.

After the addition of sulfites to foods, these compounds may be found as sulfurous acid, free inorganic sulfites, and a large variety of bound sulfite forms. It is recognized that the combined forms of sulfite do not have antimicrobial activity. In raw sausages, where the pH can vary between 5.8 and 7.0, the preservative effect is due to the bisulfite and sulfite ions, because sulfur dioxide in molecular form practically does not exist (*8*, *9*). With regard to the bound forms, differentiation must be made between those that are easily dissociable (reversibly bound forms) and those denominated irreversible, which are very stable compounds. The *S*-sulfonates ($R-S-SO_3^-$), which are formed by reaction between the sulfites and the disulfide bonds of cystine, peptides, and proteins, are considered to be irreversible forms (*10*−*13*).

$$RS - SR + SO_3^{2-} \rightarrow RS - SO_3^- + RS^-$$

In this reaction the lysis of disulfide bonds is produced by a nucleophilic displacement mechanism to form thiol and *S*-sulfonate compounds.

In sulfited foodstuffs the proportion of sulfite bound to proteins is practically unknown, due, in great part, to the fact that with the methods habitually used for the analysis of sulfite this fraction is not determined. As far as we know, only the results obtained in model assays are available (*14*−*16*) and those of the contents determined in commercial shrimps in our laboratory (*17*). In model assays we have found that there is a limited degree of formation of *S*-sulfonates and that neither the sulfite nor the cystine appears to be the limiting factor of the interaction (*14*). The reduction of the disulfide bonds modifies the functional properties of the proteins and can improve the quality of meat products (*18*, *19*).

Although the sulfites have a long history of safe use in meat products, at present, there is a tendency to restrict their use both in these products and in other foodstuffs. This is due, in part, to the appearance of adverse reactions in sensitive individuals after consuming certain sulfited foodstuffs (*12*, *20*−*23*).The appearance, or not, of adverse effects depends on the sensivity threshold of the individual, the type of food involved, and the residual levels of SO_2 (*12*, *20*, *24*). Some researchers are trying to develop novel preservation systems for meat products (raw pork sausages), but according to their results the utilization of sulfites cannot be totally discarded, although they manage to reduce its quantity by combining it with chitosan (*25*).

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Table 1. Content of *S*-Sulfonates in Raw Meat Products

burgers				sausages			
beef		chicken		beef		chicken	
total sulfite[a] (SO_2 μg/g)	*S*-sulfonates (SO_2 μg/g)	total sulfite[a] (SO_2 μg/g)	*S*-sulfonates (SO_2 μg/g)	total sulfite[a] (SO_2 μg/g)	*S*-sulfonates (SO_2 μg/g)	total sulfite[a] (SO_2 μg/g)	*S*-sulfonates (SO_2 μg/g)
188	61	171	47	161	68	502	62
159	102	413	66	313	73	401	72
332	126	198	92	305	75	317	98
396	127	348	100	447	77	243	99
230	142	399	111	361	84	322	100
297	166	723	158	171	93	386	105
258	171	627	179	447	100	656	120
490	173	294	181	284	114	625	124
522	180	495	203	330	139	563	167
708	238	421	219	462	156	836	210
1260	259	552	250				

[a] Free + reversibly bound.

The aim of this work is to evaluate the content of *S*-sulfonates in commercial samples of sausages and burgers. As we have commented previously, the content of these compounds in commercial samples is unknown. As this deals with foodstuffs that must be submitted to a cooking process, it is necessary to know the influence of the process on the content of *S*-sulfonates. It is recognized that these compounds are very stable and, in principle, it could be expected that they would not be modified by cooking, but this aspect has not been studied previously.

The results would improve the knowledge of the reactivity of the additive and contribute information that should be borne in mind in the evaluation of exposure to sulfites (*26*−*29*).

MATERIALS AND METHODS

Apparatus. A Milton Roy model CM-4000 HPLC system was equipped with an electrochemical detector (Metrohm, model 6565) with a glassy carbon electrode at 1150 mV and an Ag/AgCl reference electrode. Output from the detector was fed to a Milton Roy model CI-4000 integrator. Separation was accomplished on a 150 × 7.8 mm anion exclusion column (Waters Chromatography, Milford, MA).

Samples. The burgers and sausages were purchased from different foodstuff establishments. They were distributed according to the main meat component in groups: beef and chicken. In their elaboration a preparation was used that contained, as well as the sulfites (E-221, sodium sulfite, and/or E-224, potassium metabisulfite), salt, sugars, starch, spices, and colorants. At the time of purchase, all samples showed suitable sensory characteristics. They were stored at −30 °C until the moment of the analysis.

To evaluate the content of *S*-sulfonates, 62 raw samples were used that included burgers (42) and sausages (20). To study the influence of the cooking process, 20 samples of burgers were grilled, and the raw burgers from the same group were used as reference.

The treatment on the grill has been carried out under controlled conditions intending to reproduce the habitual form of preparation of these products. It was always performed individually, at the same temperature (150 °C) and with a total cooking time of 12 min. They were then left to cool for half an hour, and the determination of free SO_2, total SO_2, and *S*-sulfonates was then made. With the objective of the comparison of the concentrations of the additive found in raw and grilled burgers, the values determined in these latter are referred to raw weight. For this an individual correction factor was applied that is the quotient of the weights of the samples after and before cooking.

Procedure for the Determination of *S*-Sulfonates. The method set up in our laboratory was utilized (*14*). This method includes the treatment of an aliquot with potassium cyanide at pH 10 to release the sulfite bound to proteins. At the same time, another aliquot of the same sample was subjected to identical treatment but without cyanide. Then, SO_2 was determined in both aliquots by HPLC with electrochemical detection (*30*). In the samples treated with cyanide, free sulfite plus the reversibly bound form and also that bound to proteins were quantified. In the other aliquot, only the total sulfite (free plus reversibly bound) was determined. The protein-bound sulfite was calculated from the difference between the sulfite contents determined in both aliquots.

Table 2. Content of Sulfite in Raw and Grilled Burgers

raw			grilled		
free sulfite (SO_2 μg/g)	total sulfite[a] (SO_2 μg/g)	*S*-sulfonates (SO_2 μg/g)	free sulfite (SO_2 μg/g)	total sulfite[a] (SO_2 μg/g)	*S*-sulfonates (SO_2 μg/g)
Beef Burger					
128	189	65	82	145	48
175	386	106	126	302	71
152	485	119	119	363	67
366	506	119	252	334	62
213	460	124	158	325	95
133	207	125	102	139	69
105	194	136	82	150	102
115	230	142	81	163	109
243	529	217	160	410	133
304	453	267	196	294	181
Chicken Burger					
100	202	63	73	159	49
363	465	100	273	362	85
292	478	102	195	353	81
264	377	106	193	215	63
89	236	109	70	189	87
344	756	138	268	515	101
246	406	139	188	321	81
312	419	140	227	266	84
396	747	204	237	494	159
321	430	233	178	347	164

[a] Free + reversibly bound.

Procedure for the Determination of Free and Total Sulfite. The method involved the extraction of the free sulfite with a 0.050 M solution of sulfuric acid containing 0.1% (v/v) glycerol. For the extraction of the total sulfite we used 0.020 M Na_2HPO_4 adjusted to pH 10 with NaOH, also containing 0.1% (v/v) glycerol. Afterward, the determination of the sulfite was performed by HPLC as described previously (*30*).

Statistics. An *F* test for comparison of standard deviations and a *t* test for comparison of means were used (*31*).

RESULTS AND DISCUSSION

The concentrations of *S*-sulfonates and total SO_2 determined in burgers and sausages of beef and chicken are presented in **Tables 1** and **2**. Although it is not the objective of this work, we wish to comment that 18% of the samples exceeded the

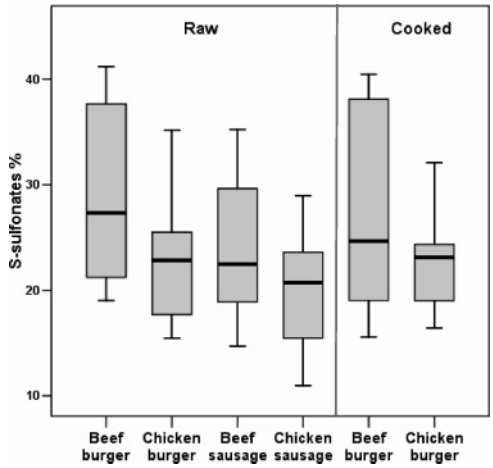

Figure 1. Percentages of *S*-sulfonates regarding the determined sulfite (SO_2 total + *S*-sulfonatos) in the types of meat products analyzed. Box plot: the box is limited by the percentiles 25 and 75 (Turkey hinges), and the inner line corresponds to the median value. The whiskers are the maximum and minimum observed values.

maximum permissible value of total sulfite (free plus reversibly bound). When the concentrations determined were close to 450 μg of SO_2 total/g, we have calculated expanded uncertainties. The value obtained by subtracting the uncertainty from the reported concentration is used to assess compliance, in accordance with the recommendations of the European Union with regard to the treatment of analytical variability in the interpretation of statutory limits (*32*).

In earlier works we found that ~60% of the samples surpassed the reference value and some particular samples even had concentrations of SO_2 between 8 and 16 times that level (*7*, *30*). It is probable that the implementation of programs of monitoring and periodical control by the responsible health officials has contributed to the improvement observed.

In relation to the *S*-sulfonates, the levels determined range between 47 and 267 μg of SO_2/g, there being a relationship with the concentrations of total sulfite, although a good statistical correlation could not be established. The results are shown graphically in **Figure 1**. This figure uses box plots to show the different percentages of *S*-sulfonates with regard to the sulfite determined (total SO_2 plus *S*-sulfonates). It can be seen that a great dispersion exists in the results, and this can be attributed, in part, to the possible differences in composition and the conditions of preparation of the samples, given that we are dealing with commercial burgers and sausages. Despite that, the degrees of formation of *S*-sulfonates are similar in all of the meat products, and a tendency to somewhat higher values in beef burgers is observed, although it is in these samples that a greater dispersion is found.

In assays carried out with sulfited meat in our laboratory, we found that the proportion of *S*-sulfonates remained relatively constant and was not conditioned by the meat component, the level of addition of sulfite, or the fat content. Nonetheless, these two last factors are inversely correlated with the retention of sulfite in the foodstuff (*14*). In beef and chicken meats to which 600 μg of SO_2/g was added, the percentages of formation of *S*-sulfonates were 15 and 22% and are lower than those found in the commercial samples (*14*). A differential characteristic of these last that could affect the formation of *S*-sulfonates is the presence of salt incorporated for the preparation of meat products. The effect of NaCl on the proteins would explain the increase in *S*-sulfonates in the commercial samples in relation to the corresponding model assays. It is possible that ionic linkages of the protein structure might be ruptured by salts; the protein would then be partially unfolded, and the buried disulfide bonds could be exposed to sulfite (*33*).

Table 3. Reduction of Sulfite in Grilled Burgers

	parameter	free SO_2	total SO_2	*S*-sulfonates
beef burgers	$\bar{X}$, %	29	28	34
	SD	5.5	5.1	9.4
	n	10	10	10
chicken burgers	$\bar{X}$, %	29	28	28
	SD	7.8	8.3	9.7
	n	10	10	10
				$F = 1.065$
				$t = 2.101$

Other authors find initial losses of sulfite in raw sausages that correspond to 26% of the added sulfite, which they attribute to the oxidation of the additive and to the formation of irreversibly bound forms (*1*). These losses have been calculated from the difference between the sulfite added and that determined, the latter not including the protein-bound fraction. Roller et al. (*25*) found that in the presence of chitosan the sulfite decreases less rapidly in the first 3 days of storage of refrigerated sausages. They attribute this effect to a reversible interaction between both additives, which would protect the sulfite from degradation. Again, in this work, the contents of sulfite refer only to the total sulfite (free sulfite plus reversibly bound) and, therefore, the possibility of the additive binding to the proteins has not been considered. The formation of *S*-sulfonates in frozen shrimps is much more variable, but, as we have discussed in previous works, this variability could be due, among other factors, to the form of application of additive. There is also the possibility that in frozen storage of these products cleavages and rearrangements of disulfide bonds are produced that could favor the formation of sulfonates (*17*, *34*).

To evaluate the influence of the cooking process on the contents of SO_2 in burgers, 20 samples prepared on the grill have been studied, using raw burgers from the same batch as reference. In **Table 2** the contents of free and total SO_2 and *S*-sulfonates in raw and grilled burgers of beef and chicken are presented. The results obtained show that cooking on the grill always causes significant losses in the levels of sulfite in any of its forms. In absolute amounts, the values of losses vary between 19 and 159 μg of free SO_2/g, between 43 and 253 μg of total SO_2/g, and between 14 and 86 μg of *S*-sulfonates/g, although the minor quantities do not always correspond to these compounds. Given the stability of the *S*-sulfonates, it appeared to be probable that when the meat products were submitted to cooking only the free or reversibly bound forms would be affected and the formation of irreversible compounds would even be favored. In fact, this is one of the theoretical reasons commonly adduced to justify, at least in part, the reduction in the levels of sulfite in cooked foodstuffs. It is possible that during the cooking cleavages of some compounds that release SO_2 occur and new adducts are formed, but the net effect is the decrease in all of the forms without there being substantial modifications in their distribution. With regard to the relationship between *S*-sulfonates and determined sulfite in the cooked samples (**Figure 1**) the same tendency as in the raw samples is observed, the median values being similar in all of them.

When the percentages of reduction for the different samples are considered (**Table 3**), it is found that they are independent of the concentration of sulfite present and of the meat component employed in the elaboration of the burgers with no significant differences being found for the distinct forms of sulfite. Thus,

the mean percentages of reduction and their deviations have been calculated for the total of the samples. The value corresponding to the *S*-sulfonates is 31 ± 9.8%. In the case of free sulfite it is 29 ± 6.6%, being very similar to the value for total sulfite that represents a percentage of 28 ± 6.7%. Both values are inferior to those found when the meat derivatives are submitted to a process of frying (*7*). Moreover, with this treatment a greater dispersion among the results is observed, due to the possible interactions among the components of the foodstuff and those of the oil used and the variations in the degree of lipidic absorption, aspects that do not occur in grilling.

From the results found in this work it can be concluded that in raw meat products the degree of formation of *S*-sulfonates is similar in all of the samples and is not conditioned by the major meat component or by the type of product (burger or sausages).

The grilling process of burgers causes significant losses in the contents of the additive in any of its forms, and the percentage of reduction of the theoretically more stable forms, *S*-sulfonates, is of the same order as that of other forms of the additive.

LITERATURE CITED

(1) Banks, J. C.; Dalton, H. K.; Nychas, G. J.; Board, R. G. Sulfite, an effective agent in the microbiological and chemical changes occurring in uncooked comminuted meat products. *J. Appl. Biochem.* **1985**, *7*, 161−179.

(2) IFST. *Presevatives in Food*; The Institute of Food Science and Technology: London, U.K., 1988; pp 1−28.

(3) Walker, R. Sulphiting agents in foods: some risk/benefit consideration. *Food Addit. Contam.* **1985**, *2*, 5−24.

(4) Bover-Cid, S.; Miguélez-Arrizado, M. J.; Vidal-Carou, M. C. Biogenic amine accumulation in ripened sausages affected by the addition of sodium sulphite. *Meat Sci.* **2001**, *59*, 391−396.

(5) Wedzicha, B. L. Uses in food. In *Chemistry of Sulphur Dioxide in Foods*; Elsevier Applied Science: London, U.K., 1984; pp 275−311.

(6) Parlamento Europeo y Consejo de la Unión Europea. Directiva 95/2/CE del Parlamento Europeo y del Consejo de 20 de Febrero de 1995 relativa a aditivos alimentarios distintos de los colorantes y edulcorantes. *Off. J. Eur. Communities* **1995**, *L61* (March 18), 1−40.

(7) Armentia-Alvarez, A.; Fernández-Casero, A.; García-Moreno, C.; Peña-Egido, M. J. Residual levels of free and total sulphite in fresh and cooked burgers. *Food Addit. Contam.* **1993**, *10*, 157−165.

(8) Banks, J. G.; Board, R. G. Comparison of methods for the determination of free and bound sulphur dioxide in stored British fresh sausage. *J. Sci. Food Agric.* **1982**, *33*, 197−303.

(9) Wedzicha, B. L. Role of sulphur dioxide. *Food Sci. Technol. Today* **1987**, *1*, 169−171.

(10) Daniels, D. H.; Warner, C. R.; Joe, F. L., Jr. Colorimetric determination of sulfite in vegetables with modified *p*-rosaniline procedure. Presented at the 192nd National Meeting of the American Chemical Society, Los Angeles, CA, 1986.

(11) Fazio, T.; Warner, C. R. A review of sulphites in foods: analytical methodology and reported findings. *Food Addit. Contam.* **1990**, *7*, 443−454.

(12) Taylor, S. L.; Higley, N. A.; Bush, R. K. Sulfites in foods: uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity and hypersensitivity. *Adv. Food Res.* **1986**, *30*, 1−75.

(13) Wedzicha, B. L. Chemistry of sulphiting agents in food. *Food Addit. Contam.* **1992**, *9*, 449−459.

(14) García-Alonso, B.; Peña-Egido, M. J.; García-Moreno, C. *S*-Sulfonate determination and formation in meat products. *J. Agric. Food Chem.* **2001**, *49*, 423−429.

(15) Thewlis, B. H.; Wade, P. An investigation into the fate of sulphites added to hard sweet biscuit doughs. *J. Sci. Food Agric.* **1974**, *25*, 99−105.

(16) Wedzicha, B. L.; Mountfort, K. A. Reactivity of sulphur dioxide in comminuted meat. *Food Chem.* **1991**, *39*, 281−297.

(17) Armentia-Alvarez, A.; Peña-Egido, M. J.; García-Moreno, C. *S*-Sulfonate determination in shrimp. *J. Agric. Food Chem.* **1997**, *45*, 791−796.

(18) Friedman, M. Improvement in the safety of foods by SH-containing amino acids and peptides. A review. *J. Agric. Food Chem.* **1994**, *42*, 3−20.

(19) Ramezani, R.; Aminlari, M.; Fallahi, H. Effect of chemically modified soy proteins and ficin-tenderized meat on the quality atributes of sausage. *Food Chem. Toxicol.* **2003**, *68*, 85−88.

(20) Bush, R. K.; Zoratti, E.; Taylor, S. L. Diagnosis of sulfite and aspirin sensitivity. *Clin. Rev. Allergy* **1990**, *8*, 159−178.

(21) EFSA (European Food Safety Authority). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes (request EFSA-Q-2003-016). Adopted on 19 February 2004. *EFSA J.* **2004**, *32*, 1−197.

(22) FAO/WHO. Preservative: Sulfur dioxide and sulfites. Assessments of the intake of specific food additives: sulfites. In *Safety Evaluation of Certain Food Additives*; WHO Food Additives Series *42*; World Health Organization: Geneva, Switzerland, 1999; pp 95−116, 451−452.

(23) Simon, R. A. Adverse reactions to food and drug additives. *Inmmunol. Allergy Clin.* **1996**, *16*, 137−176.

(24) Prieto, L.; Juyol, M.; Paricio, A.; Martínez, M. A.; Palop, J.; Castro, J. Oral challenge test with sodium metabisulfite in steroid-dependent asthmatic patients. *Allergol. Immunopathol.* **1988**, *16*, 393−396.

(25) Roller, S.; Sagoo, S.; Board, R.; O'Mahony, B.; Caplice, E.; Fitzgerald, G.; Fogden, M.; Owen, M.; Fletcher, H. Novel combinations of chitosan, carnocin and sulphite for the preservation of chilled pork sausages. *Meat Sci.* **2002**, *62*, 165−177.

(26) Gibson, W. B.; Strong, F. M. Accumulation of ingested sulphite and sulphate-sulphur and utilization of sulphited proteins by rats. *Food Cosmet. Toxicol.* **1974**, *12*, 625−640.

(27) Gibson, W. B.; Strong, F. M. Metabolism and elimination of α-hydroxyethane sulphonate by rats. *Food Cosmet. Toxicol.* **1976**, *14*, 41−43.

(28) Gunnison, A. S.; Palmes, E. D. Persistence of plasma *S*-sulfonates following exposure of rabbits to sulfite and sulfur dioxide. *Toxicol. Appl. Pharmacol.* **1973**, *24*, 266−278.

(29) Ribera, D.; Jonker, D.; Narbonne, J. F.; O'Brien, J.; Antignac, E. Absence of adverse effects of sodium metabisulphite in manufactured biscuits: results of subacute (28-days) and subchronic (85-days) feeding studies in rats. *Food Addit. Contam.* **2001**, *18*, 103−114.

(30) Paino-Campa, G.; Peña-Egido, M. J.; García-Moreno, C. Liquid chromatographic determination of free and total sulphites in fresh sausages. *J. Sci. Food Agric.* **1991**, *56*, 85−93.

(31) Miller, J. C.; Miller, J. M. Basic statistical methods for analytical chemistry. Part I. Statistics of repeated measurements. A review. *Analyst* **1988**, *113*, 1351−1356.

(32) DG Health & Consumer Protection. Report on the relationship beween analytical results, measurement uncertainty, recovery factors and the provisions of EU Food and Feed legislation, with particular reference to community legislation concerning. July 2004, 36 pp. http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf

(33) Link, T. M.; Park, J. W. Solubility of salmon myosin as affected by conformational changes at various ionic strenghs and pH. *J. Food Sci.* **1998**, *63*, 215−218.

(34) Armentia-Alvarez, A.; García-Moreno, C.; Peña-Egido, M. J. Residual levels of sulfite in raw and boiled frozen shrimp: variability, distribution and losses. *J. Food Prot.* **1994**, *57*, 66−69.

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