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Monitoring of residual sulfur dioxide content in pickled mushroom sold in the State of São Paulo from 2016 to 2022

Monitoramento do teor residual de dióxido de enxofre em cogumelo em conserva comercializado no estado de São Paulo no período de 2016 a 2022

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ABSTRACT

Introduction: Sulfiting agents have many technological functions: they act as preservatives, antioxidants, flour treatment agents, whiteners and control enzymatic and non-enzymatic browning. In mushrooms, sulfite acts by inhibiting the enzymatic action of polyphenoloxidase, delaying oxidative browning. The presence of sulfites in foods has been linked to adverse reactions in sensitive people. The established value for acceptable daily intake (ADI) is 0 – 0.7 mg/kg body weight, expressed as sulfur dioxide (SO_2). RDC No. 8, of March 6, 2013, of the National Health Surveillance Agency of the Ministry of Health established a maximum limit of 0.005 g/100 g (50 mg/kg) for pickled mushrooms as residual SO_2 in the antioxidant function. **Objective:** To determine the residual SO_2 content in pickled mushrooms and evaluate the additive declaration on the label. **Method:** From 2016 to 2022, 57 samples from 28 different brands of pickled mushrooms were shared by the Health Surveillance Offices. The determination of total SO_2 followed the optimized Monier-Williams distillation method. **Results:** Of the samples evaluated, 33 (58%) presented unsatisfactory SO_2 content, above the maximum limit established in the specific technical regulation. High levels were found, reaching 2,591 mg/kg (0.2591 g/100 g). Six unsatisfactory brands in terms of SO_2 contents were also in disagreement because they did not declare the addition of the additive in the list of ingredients on the label, which could represent a serious risk for individuals sensitive to sulfites. **Conclusions:** The high percentage of samples in disagreement with the maximum limit of the additive established in the legislation and the non-declaration in the list of ingredients on the label, indicate the need for a greater control of the producers and distributors in the use of sulfite salts and for a continuity of monitoring of SO_2 content in pickled mushrooms.

KEYWORDS: Sulfite; Sulfur Dioxide; Monier-Williams Method; Pickled Mushrooms; Champignon

RESUMO

Introdução: Agentes sulfitantes apresentam muitas funções tecnológicas: atuam como conservadores, antioxidantes, agentes de tratamento de farinhas, branqueadores e controlam o escurecimento enzimático e não enzimático. Nos cogumelos, o sulfito age inibindo a ação enzimática da polifenoloxidase, retardando o escurecimento oxidativo. A presença de sulfitos em alimentos tem sido relacionada a reações adversas em indivíduos sensíveis. O valor estabelecido para ingestão diária aceitável (IDA) é de 0 – 0,7 mg/kg de peso corpóreo, expresso como dióxido de enxofre (SO_2). A RDC nº 8, de 6 de março de 2013 da Agência Nacional de Vigilância Sanitária do Ministério da Saúde estabeleceu para cogumelos em conserva o limite máximo de 0,005 g/100 g (50 mg/kg) como SO_2 residual na função antioxidante. **Objetivo:** Determinar o teor de SO_2 residual em cogumelos em

conserva e avaliar a declaração do aditivo no rótulo. **Método:** No período de 2016 a 2022 foram encaminhados pelas Vigilâncias Sanitárias 57 amostras de 28 diferentes marcas de cogumelos em conserva. A determinação de SO_2 total seguiu o método de destilação de Monier-Williams otimizado. **Resultados:** Das amostras avaliadas, 33 (58%) apresentaram teor de SO_2 insatisfatório, acima do limite máximo estabelecido no regulamento técnico específico. Foram encontrados elevados teores atingindo 2.591 mg/kg (0,2591 g/100 g). Seis marcas insatisfatórias pelos teores de SO_2 também estavam em desacordo por não declararem a adição do aditivo na lista de ingredientes do rótulo, podendo representar sério risco para os indivíduos sensíveis aos sulfitos. **Conclusões:** A elevada porcentagem de amostras em desacordo com o limite máximo do aditivo estabelecido na legislação e a não declaração na lista de ingredientes do rótulo indicam a necessidade de um maior controle dos produtores e distribuidores no uso de sais de sulfito e continuidade do monitoramento do teor de SO_2 em cogumelos em conserva.

PALAVRAS-CHAVE: Sulfito; Dióxido de Enxofre; Método Monier-Williams; Cogumelos em Conserva; Champignon

INTRODUCTION

Edible mushrooms are fungi of great commercial importance, consumed for thousands of years for their nutritional quality and therapeutic effects. In Brazil, the main edible species cultivated are: *Agaricus bisporus* (Paris mushroom), *Pleurotus ssp* (Shimeji), *Lentinula edodes* (Shiitake), and *Agaricus blazei* Murrill (Cogumelo do Sol)[1,2]. According to the Brazilian National Association of Mushroom Producers - ANPC (2013), the most cultivated species in Brazil is *A. bisporus,* with an estimated production of 8,000 tons/year, corresponding to 66% of all *fresh* mushrooms produced *in the* country[2], and is also the most cultivated *in* the world.

These foods are highly perishable and suffer a rapid loss of quality after harvesting. They should be consumed within 1 to 3 days if kept at room temperature, and 5 to 7 days when stored at 2°C[3]. Due to their short shelf life, 55% are processed, with 5% in dehydrated form and 50% in pickled form[4]. Their quality is determined by color, texture, cleanliness, color, texture, aroma and flavor, but color is the first attribute perceived by consumers, and browning is the main factor in loss of quality, affecting acceptance and marketing[4,5,6]. This enzymatic browning after harvest is a complex process caused by the oxidation of phenolic groups, catalyzed by the action of the enzyme polyphenol oxidase (PFO)[4,6]. To inhibit the intense activity of PFO in mushrooms, the most widely used method is the addition of sulphiting agents[7,8].

Sulphites or sulphiting agents refer to sulphur dioxide gas (SO_2) and several various inorganic sulphite salts, which release SO_2 under conditions of use. They are widely used as food additives due to their low cost, effectiveness, and variety of technological functions: antioxidant, controlling enzymatic and non-enzymatic browning reactions, modifying the structure and functional properties of protein, preservative, enzyme inhibitor, bleaching agent, and flour treatment agent[9,10,11].

Despite its multiple technological functions, ingestion of SO_2 can cause adverse effects in sensitive people, such as: difficulty breathing, anaphylactic shock, headache, nausea, gastric irritation, urticaria, and induction of asthmatic reactions[7,8,10,12]. Sulfiting agents have been evaluated by the *Joint FAO/WHO Expert Committee on Food Additives* (JECFA)[13], which established in 1974 the group acceptable daily intake (ADI) value for sulfites at 0 - 0.7 mg/kg body weight, expressed as SO_2.

In Brazil, the use of sulphites in pickled mushrooms is regulated by the Resolution of the Collegiate Board (RDC) of the Brazilian National Health Surveillance Agency (Anvisa) No. 8, of March 6, 2013[14], which authorizes the use of: sulfur dioxide (INS 220), sodium sulfite (INS 221), sodium bisulfite (INS 222), sodium metabisulfite (INS 223), potassium metabisulfite (INS 224), potassium sulfite (INS 225), calcium bisulfite (INS 227), and potassium bisulfite (INS 228). Sulphites are intended for use in pickled mushrooms as antioxidants, at a maximum limit of 0.005 g/100 g (50 mg/kg), expressed as residual SO2[14]. This limit is the same as that recommended by the *Codex* Committee on Food Additives (CCFA), specified in the *General Standard for Food Additives* (GSFA)[15] and by Directive No. 95/2/EC[16] of the European Parliament and of the Council of the European Union, which regulated the use of sulphites in processed mushrooms, including frozen mushrooms, at the same maximum limit as the Brazilian legislation of 50 mg/kg, expressed as SO_2.

In March 2023, Normative Instruction (IN) of the Anvisa Collegiate Board No. 778, of March 1, 2023[17] was published, revoking Anvisa RDC No. 8/2013, and IN of the Anvisa Collegiate Board No. 211, of March 1, 2023[18] came into force, establishing the technological functions, maximum limits, and conditions of use for food additives and technology adjuvants authorized for use in food. The limit, technological function, and sulphiting agents allowed in pickled mushrooms remain the same as in the previous legislation, only the unit of use limits for additives has been expressed in mg/kg.

In addition, ANVISA RDC No. 259, of September 20, 2002[19], made it compulsory to inform food additives in the list of ingredients on the label according to their main technological function followed by their full name and/or their number in the International Numbering System (INS) of the *Codex Alimentarius*. Anvisa's RDC No. 727/2022[20], effective as of September 1, revoked RDC No. 259/2002, without, however, changing the way in which food additives are declared in the list of ingredients.

Given this scenario and the great potential for individuals to be exposed to sulphites through food, the aim of this study

was to assess, in accordance with current legislation, the residual SO_2 content and the declaration of the additive in the list of ingredients on the label of pickled mushrooms sold in the state of São Paulo, providing the necessary data to assess exposure through the ingestion of sulphites in the consumption of pickled mushrooms.

METHOD

From 2016 to 2022, the SO_2 content and its declaration in the list of ingredients on the label of 57 samples of whole or sliced pickled mushrooms from 28 different brands marketed in the state of São Paulo, which are coded by letters of the alphabet, were evaluated. The products were packaged in glass jars or plastic film sachets (two packages or sample units per seal) and were sent to the food physicochemical analysis laboratory by the São Paulo State Health Surveillance Agency (Visa), in order to comply with the São Paulo Food Inspection Analysis Program (PP) and the Monitoring National Program for Additives and Contaminants (Promac) in food by Anvisa/Ministry of Health. Promac planning is carried out in partnership with the Visa and the Public Health Central Laboratories (Lacens), taking into account the local products of interest, their health priorities, frequency of consumption and the laboratories' analytical capacity. The PP is a joint program between the Health Surveillance Centre (CVS) and the Instituto Adolfo Lutz (IAL) and its criteria for selecting products are: health risks, foods with a high percentage of unsatisfactory analytical results (historical), suggestions made by representatives of the CVS, the Visa, and the IAL. The products and additives selected by Promac are also included in the São Paulo Program (PP).

The analytical determination of total SO_2 followed the official method of the *Association of Official Analytical Chemists* (AOAC)[21] of optimized Monier-Williams distillation, also taking as a reference the methodology verification study described by Nagato et al.[22], with a limit of quantification (LOQ) of 7 mg/kg. 50 g of mushrooms drained and chopped or ground in a domestic food processor were analyzed. Each sample unit of the product was analyzed minimally in duplicate. The average result of the analysis was expressed as residual SO_2 and calculated in mg/kg and g/100 g.

The statistical functions of the Microsoft Excel 2016 *software* were used to evaluate the mean and median SO_2 content of the samples. The variability of the results was measured by tabulating the values: minimum, maximum, standard deviation (SD - dispersion around the mean) and coefficient of variation (CV - relative dispersion or per unit of mean).

RESULTS AND DISCUSSION

Chart 1 shows the results of the residual SO_2 levels found in 57 samples of pickled mushrooms from 28 different commercial brands, as well as the declarations or not of sulphiting agents in the ingredient lists on the labels, the levels ranging from < 7 mg/kg (< LOQ) to 2,591 mg/kg. Chart 2 shows the mean (218 mg/kg) and median (84 mg/kg), revealing the high dispersion of the results by the coefficient of variation in the statistical analysis (CV = 188%) and the non-uniformity in the use of sulphiting agents in mushrooms by the companies.

Of the 57 samples, 33 (58%) showed unsatisfactory results (Figure), with residual SO_2 content above the reference value of 50 mg/kg (0.005 g/100g). High levels were found, reaching 2,591 mg/kg, with the products not complying with the legislation[14].

During the period, 28 brands were evaluated, of which 14 (E, F, I, L, M, R, U, V, W, X, Y, Z, AA, and AB) were collected once and of these only three brands (M, W, and AB) showed satisfactory results.

Brand A was sampled four times in different years and maintained an unsatisfactory result. Brands J, Q, and T were evaluated twice with unsatisfactory results. Eight brands (B, C, D, G, K, N, P, and S) presented unsatisfactory and satisfactory results, and most of them corrected the levels in the new evaluation.

Only two brands (H and O) were sampled twice with satisfactory results, complying with current legislation both in terms of SO_2 content and the declaration of this additive on the label. Also noteworthy was brand W, which did not declare the addition of this antioxidant, and its presence was not detected.

The values found emphasize the need for new sampling of the brands that showed unsatisfactory results. Of particular note was the high SO2 content found in brand Y (2,591 mg/kg), which exceeded the maximum permitted limit of 50 mg/kg by 51 times.

The declarations of sulphite when they appear in the list of ingredients on the label (Chart 1) followed the presentations authorized for food additives, i.e., by their INS number or their full name in full, or both[19].

The sulphiting agents declared in the list of ingredients were sodium metabisulphite (INS 223) and sulphur dioxide (INS 220). Seventeen brands (B, C, F, G, I, J, M, N, O, P, Q, S, T, V, Y, AA, AB) declared added sulphite by its full name (sodium metabisulphite or sulphur dioxide), and three brands (P, Q, and S) which had not declared the presence of this additive in a first collection, corrected this information in new batches.

Four brands (A, D, H, K) declared the presence of the additive by INS number. Although the technical regulation for labeling packaged foods19 authorizes additives to be declared only by INS number, not all consumers will identify the added additive, in which case the health risk is greater for individuals sensitive to sulphites.

Five brands (F, S, T, Y, and AB) declared the presence of sulphite in the preservative function, in disagreement with Anvisa/Ministry of Health RDC No. 8/2013, which authorizes the use of this additive in the antioxidant function for pickled mushrooms[14].

Six brands (E, L, R, U, X, Z) did not declare the presence of sulphites in the list of ingredients and showed levels of SO_2 above

Chart 1. Sulfur dioxide content and declaration of the additive on the label of the commercial brands of pickled mushroom evaluated from 2016 to 2022.

Year	Brand	SO_2 (mg/kg)	SO_2 (g/100g)	Conclusion[a]	Sulphite declared in the list of ingredients on the label
2016	A	**278**	**0,0278**	**Unsatisfactory**	INS 223
2016	B	**83**	**0,0083**	**Unsatisfactory**	Sulphur dioxide
2016	C	**87**	**0,0087**	**Unsatisfactory**	Sodium metabisulfite
2017	A	**129**	**0,0129**	**Unsatisfactory**	INS 223
2017	B	42	0,0042	Satisfactory	Sulphur dioxide
2017	C	47	0,0047	Satisfactory	Sodium metabisulfite
2017	C	30	0,0030	Satisfactory	Sodium metabisulfite
2017	C	35	0,0035	Satisfactory	Sodium metabisulfite
2017	D	39	0,039	Satisfactory	INS 223
2017	E	**188**	**0,0188**	**Unsatisfactory**	Undeclared
2018	A	**225**	**0,0225**	**Unsatisfactory**	INS 223
2018	B	**60**	**0,0060**	**Unsatisfactory**	Sulphur dioxide
2018	C	50	0,0050	Satisfactory	Sodium metabisulfite
2018	C	46	0,0046	Satisfactory	Sodium metabisulfite
2018	D	**147**	**0,0147**	**Unsatisfactory**	INS 223
2018	F	**1.299**	**0,1299**	**Unsatisfactory**	Sulphur [dioxideb]
2018	G	**325**	**0,0325**	**Unsatisfactory**	Sulphur dioxide
2018	G	48	0,0048	Satisfactory	Sulphur dioxide
2018	H	43	0,0043	Satisfactory	INS 220
2018	I	**63**	**0,0063**	**Unsatisfactory**	Sodium metabisulfite
2018	J	**160**	**0,0160**	**Unsatisfactory**	Sodium metabisulfite
2018	K	**440**	**0,0440**	**Unsatisfactory**	INS 220
2018	K	**370**	**0,0370**	**Unsatisfactory**	INS 220
2018	L	**648**	**0,0648**	**Unsatisfactory**	Undeclared
2018	M	49	0,0049	Satisfactory	Sodium metabisulfite
2018	N	**132**	**0,0132**	**Unsatisfactory**	Sulphur dioxide
2018	O	50	0,0050	Satisfactory	Sodium metabisulfite
2019	H	27	0,0027	Satisfactory	INS 220
2019	J	**119**	**0,0119**	**Unsatisfactory**	Sodium metabisulfite
2019	K	43	0,0043	Satisfactory	INS 220
2019	N	19	0,0019	Satisfactory	Sulphur dioxide
2019	O	18	0,0018	Satisfactory	Sodium metabisulfite
2019	P	**122**	**0,0122**	**Unsatisfactory**	Undeclared
2019	P	39	0,0039	Satisfactory	Sodium metabisulfite
2019	Q	**198**	**0,0198**	**Unsatisfactory**	Undeclared
2019	R	**559**	**0,0559**	**Unsatisfactory**	Undeclared
2019	S	**147**	**0,0147**	**Unsatisfactory**	Undeclared
2019	T	**308**	**0,0308**	**Unsatisfactory**	Sulphur dioxide[b]
2019	U	**1.045**	**0,1045**	**Unsatisfactory**	Undeclared
2019	V	**136**	**0,0136**	**Unsatisfactory**	Sulphur dioxide
2019	W	[<7c]	<0,0007[c]	Satisfactory	Undeclared

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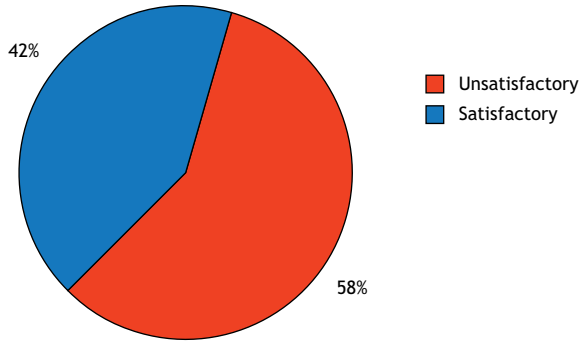
2021	X	**819**	**0,0819**	**Unsatisfactory**	Undeclared
2021	Y	**2.591**	**0,2591**	**Unsatisfactory**	Sodium metabisulfite INS 223[b]
2022	Z	**86**	**0,0086**	**Unsatisfactory**	Undeclared
2022	AA	**117**	**0,0118**	**Unsatisfactory**	Sodium metabisulfite
2022	AB	29	0,0029	Satisfactory	Sulphur dioxide[b]
2022	A	**155**	**0,0155**	**Unsatisfactory**	INS 223
2022	N	34	0,0034	Satisfactory	Sulphur dioxide
2022	P	31	0,0031	Satisfactory	Sodium metabisulfite
2022	Q	**136**	**0,0136**	**Unsatisfactory**	Sulphur dioxide
2022	S	24	0,0024	Satisfactory	Sulphur dioxide[b]
2022	S	**62**	**0,0061**	**Unsatisfactory**	Sulphur dioxide[b]
2022	S	27	0,0027	Satisfactory	Sulphur dioxide[b]
2022	S	17	0,0017	Satisfactory	Sulphur dioxide[b]
2022	S	**58**	**0,0058**	**Unsatisfactory**	Sulphur dioxide[b]
2022	S	32	0,0032	Satisfactory	Sulphur dioxide[b]
2022	T	**84**	**0,0084**	**Unsatisfactory**	Sulphur dioxide[b]

Source: Prepared by the authors, 2022.
[a] Content found in the analysis satisfactory or unsatisfactory according to the maximum limit of 0.005 g/100 g (as residual SO2) established in Anvisa RDC No. 8/2013[14];
[b] Additive declared in the conservative function;
[c] LOQ - Limit of quantification of 7 mg/kg or 0.0007 g/100 g (Nagato et al., 2013)[22].
INS 220: sulphur dioxide; INS 223: sodium metabisulphite.

Chart 2. Maximum, minimum, mean, median, standard deviation, and coefficient of variation values of the sulphur dioxide levels found in pickled mushrooms.

	SO_2 content	
	SO_2 (mg/kg)	SO_2 (g/100g)
Minimum	<7	<0,0007
Maximum	2591	0,2591
Mean	218	0,0218
Median	84	0,0084
SD	410	0,0410
CV (%)	188	188

Source: Prepared by the authors, 2022.
SD: standard deviation; CV: coefficient of variation.



Source: Prepared by the authors, 2022.

Figure. Percentage of satisfactory and unsatisfactory results for residual sulfur dioxide content in commercial pickled mushroom samples evaluated from 2016 to 2022 in the São Paulo State Food Inspection Analysis Program.

the maximum established limit, thus being in disagreement with the technical regulations on the use of additives[14] and the labeling of packaged foods19, posing a serious risk to the health of sensitive individuals.

As the ADI for sulfite is 0 - 0.7 mg/kg body weight expressed as SO_2, a 60 kg adult could ingest up to 42 mg of SO_2 per day over the course of their life, and a 30 kg child up to 21 mg of SO_2, without posing any appreciable health risk, based on the toxicological studies available at the time of the evaluation[13]. Considering only the intake of SO_2 from pickled mushrooms and calculating on the highest value found of 2,591 mg/kg of SO_2, a 60 kg adult could consume up to 16 g of mushrooms to reach the maximum that could be ingested per day of SO_2 and a 30 kg child only 8 g. For the average amount found, which was 218 mg/kg, a 60 kg adult could eat up to 193 g of mushrooms and a 30 kg child a maximum of 96 g. This calculation only takes into account the ingestion of this food, but due to their multiple functions, sulphites are added to a wide variety of foods, making it necessary to strictly control their use and residual content in authorized foods.

A high percentage of unsatisfactory results was reported in 2001 by Bragagnolo et al.[23], who evaluated the SO_2 content of 25 commercial samples of pickled mushrooms and found

that 68% of the products had levels higher than 0.005 g/100 g (50 mg/kg), with levels ranging from not detected (< 0.15 mg/kg) to 1,052 mg/kg. In the study by Nagato et al.[22], of six samples analyzed, three (50%) were unsatisfactory, reaching a maximum value of 1,130 mg/kg. Martins et al.[24] reported the results of Promac 2014, revealing non-compliance for five (83%) samples of pickled mushrooms, out of a total of six analyzed, whose levels ranged from 36 to 325 mg/kg. In Brazil, in addition to there being few scientific articles quantifying sulphites in pickled mushrooms, these have presented a small number of samples, and there is a need to update the scenario with a larger number of sampling units.

Sulphite levels can vary depending on the processing technology used, the chemical nature of the food, the added content, the permeability of the packaging and the storage time and temperature conditions[10,25] but the additive limit must be complied with from the start of the food's availability to the consumer.

Due to the low ADI value and the widespread use of sulphites in foods and the recurrence of unsatisfactory analytical results, it is necessary to continue monitoring the SO_2 content in pickled mushrooms, as the problem has not yet been solved. It is necessary to plan the collection of samples by Visa, giving priority to brands that presented unsatisfactory results in a single collection, repeat offenders and brands that have not yet been evaluated, applying, where appropriate, the sanctions provided for in Law No. 6,437 of August 20, 1977[26], which vary from warning, seizure and rendering useless, interdiction, cancellation of registration, and/or fining the company so that this infraction is not repeated. The competent bodies must apply the sanctions provided for and monitor the corrections of the irregularities mentioned.

CONCLUSIONS

The prevalence of high levels of SO_2 in pickled mushrooms and their non-declaration in the list of ingredients on the label highlights a public health concern, and calls for: better industrial quality control, the adoption of good processing practices, the use of technologies to control enzymatic browning, the addition of other antioxidant additives, greater enforcement action and continuity in additive monitoring programs, contributing to the safety of marketed products, and reducing the potential risk to consumer health, especially for sensitive asthmatics and children.

The monitoring results contribute to providing the necessary data to assess exposure to sulfites in the consumption of pickled mushrooms by the Brazilian population.

Although the darker appearance of the mushrooms may be less attractive to the Brazilian consumer, low levels of sulphur dioxide associated with Good Manufacturing Practices would result in safe products and best sensorially evaluated in terms of aroma and flavor.

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Authors' Contribution

Martins MS - Conception, planning (study design), analysis, data interpretation, and writing of the work. Della Torre JCM - Planning (study design), analysis, data interpretation, and writing of the work. Barbosa J - Data analysis and interpretation. All the authors approved the final version of the work.

Conflict of Interest

The authors inform that there is no potential conflict of interest with peers and institutions, political or financial, in this study.