Enzyme Engineering
ISSN: 2329-6674

Santos et al., Enz Eng 2016, 5:3
DOI: 10.4172/2329-6674.1000156

Research Article OMICS International

Quality and Occurrence of Mycotoxins in Tomato Products in the Brazilian Market

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Abstract

The study aimed at evaluating physical, chemical and microbiological quality of tomato products and to investigate the occurrence of *Alternaria alternata* mycotoxins in tomato pulp, extract and ketchup. Tomato products were evaluated for physical and chemical characteristics, as well as for the occurrence of foreign matter, quality of raw material (using the Howard mold count - HMC), and adequacy of microbiological parameters to the current Brazilian legislation. *A. alternata* mycotoxins alternariol (AOH) and alternariol monomethyl ether (AME) were quantified using High Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). Mycotoxin contamination was observed in one tomato brand commercialized in Brazil. Pulp sample from brand A presented soluble solids contents lower than 6%. Only tomato extract brand B showed no foreign material. Mycotoxins were not found in pulp and tomato paste in all brands. AOH levels ranging from 1.22 to 8.45 µg/g were found in brand A ketchup samples. Mycotoxin AME was identified in brand C ketchup. All products showed differences in physical and chemical characteristics but within the parameters described in current legislation. Regarding microbiological quality, all brands and products (paste, pulp and ketchup) are also in accordance with the legislation. Insect fragments, mites and rodent hair were identified in almost all brands and products, within acceptable limits. AOH and AME mycotoxins produced by *Alternaria alternata* were identified only in ketchups.

Keywords: Alternariol; Alternariol monomethyl ether; Quality; Tomato products; Brazilian markets

Introduction

Fungi are a major cause of reduction in agricultural yields and may contaminate food before, during and after harvest. Damage due to mycotoxins-producing fungi (secondary metabolites) goes beyond damage to fruit and may seriously compromise the quality of processed products, posing risks to food safety. In tomato fruits, *Alternaria* sp. is the main pathogen attacking fresh tomatoes [1,2].

Infection of tomatoes by *Alternaria alternata* is linked to injuries or to plant tissue fragility. In addition, other factors such as damages and the presence of free water due to rain, dew and excessive irrigation may induce the germination of spores on fruit surface. This fungus can penetrate fruit skin through injuries caused by mishandling, insect attacks and by calix scars. Measures to control production and growth of mycotoxins produced by *A. alternata* after harvest include maintaining products at temperatures below 7°C and storage periods shorter than ten days. Although the consumption of fresh tomatoes contaminated by *A. alternaria* is unlikely, their use in processing is a reality [3].

Several studies have reported the presence of mycotoxins produced by *A. alternata* in tomato products such as tenuazonic acid (TeA), alternariol monomethyl ether (AME) and alternariol (AOH) in tomato pulp samples commercialized in Argentina and in samples of pulp and tomato paste in Brazil. In tomatoes incubated for 21 days at 21°C, *A. alternata* produced five mycotoxins (AOH, AME, TeA, altuene and altertoxin-I) at several concentrations. The industrial processing of tomato products, such as heating, pasteurization, sterilization and storage has no significant effect in reducing these mycotoxins in the final product [4-7].

Alternaria sp. can produce a series of toxic and nontoxic secondary metabolites. A brief summary of the numerous secondary metabolites of *Alternaria sp.* and their toxicity is followed by a presentation of the current view of the polyketide biosynthetic mechanism and its application to the biosynthesis of these compounds. Possible mechanisms for the biosynthesis of alternariol, alternariol methyl ether, and other dibenzo-α-pyrones are discussed in different papers, as well as mechanisms and enzymes involved in the biosynthesis of tenuazonic acid and altertoxin I. Bioregulation of the production of these materials by light, heat, nutrients and NADPH production, and the role of mannitol in NADPH formation are also explored in different publications.

Mycotoxins are hard to define and to classify. It can be explained to their diverse chemical structures and biosynthetic origins. Their biosynthetic origins are polyketides, amino acid-derived, among other compounds. Recently, the tenuazonic acid (TeA) biosynthetic gene was identified from *Magnaporthe oryzae*. TeA has been detected in various Alternaria-contaminated crops, fruits and vegetables. TeA is the most toxic of the Alternaria toxins. It inhibits protein biosynthesis on ribosomes by suppressing the release of new protein. It also reportedly shows biological properties including antitumor, antibacterial, antiviral and phytotoxic activities.

Mycotoxins produced by *A. alternata* have an acute low toxic effect, but AOH and AME are mutagenic in mammalian cells. A potential estrogenic effect is assigned to AOH, depending on its potential for cell proliferation inhibition and its genotoxic effect on mammalian cells culture [8,9]. Contamination of food by *A. alternata* is one of the

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Received November 23, 2016; **Accepted** November 25, 2016; **Published** March 15, 2017

Citation: Santos GG, Mattos LM, Moretti CL (2016) Quality and Occurrence of Mycotoxins in Tomato Products in the Brazilian Market. Enz Eng 5: 156. doi:10.4172/2329-6674.1000156

etiological factors responsible for the high incidence of esophageal cancer in the province of Linxian, in China [10-12].

Since Brazil is the seventh largest grower of processing tomatoes, the consumption of fresh tomatoes decreased during the last years, as well as the consumption of processed tomato increased between 2002 and 2012 [13], the evaluation of mycotoxins of *A. alternata* in tomato products is of paramount importance. Although the risk of chronic intake of mycotoxins is a genuine treat, according to the *European Mycotoxins Awareness Network* [14,15], there are no specific regulations for any *Alternaria* produced mycotoxin in food [16]. Based on the above mentioned scenario, the present study aimed at evaluating physicochemical and microbiological quality of three different tomato products and detect the presence of *Alternaria alternata* mycotoxins.

Material and Methods

Sample selection

Samples of tomato paste (n=16), tomato pulp (n=16), ketchup bottle (n=16) and ketchup packets single-serve (n=16) were collected from local market in Brasilia, Distrito Federal, Brazil. Samples were homogenized in a digital homogenizer "stomacher" (Stomax, São Paulo, Brazil) and stored at -20°C in air tight containers prior to quality and mycotoxin determination.

Physicochemical quality evaluation

Moisture content analyses in tomato products were carried out through direct heating in an air forced oven at 75°C (Quimis, São Paulo, Brazil) to constant weight. Titratable acidity was determined by titration with sodium hydroxide (NaOH) 0.1 mol/L solution. pH was determined using a pHmeter (SI Analytics, Sao Paulo, Brazil). Total soluble solids (°Brix) was determined at 25°C with a digital bench refractometer (Atago PAL-1, Sao Paulo, Brazil), previously calibrated with distilled water [17].

Microscopic analyses

Samples were analyzed for the presence of insect fragments, mites and rodent hair in 200 g of ketchup, 100 g of paste and 100 g of pulp using the of the *Association of Official Analytical Chemists* (AOAC, 2000) 972.32 flotation method. For filament counting the 16.17.01 / 984.29 methods was used [18].

Microbiological analyses

Tomato products were also analyzed for levels of contamination by fecal coliforms (45°C), coagulase-positive staphylococci and *Salmonella* sp. presence, following the parameters and microbiological standards set by the Brazilian legislation [19].

Sample preparation

Mycotoxins extraction: The extraction procedure for *Alternaria* mycotoxins was adapted from Motta and Soares [7]. Briefly, 50 g were homogenized with 150 mL of HPLC grade methanol (J.T. Baker, Pennsylvania, USA) and filtered through quantitative filter paper (Nalgon, Sao Paulo, Brazil). An aliquot of 200 mL of filtrate was collected in a beaker and 60 mL of ammonium sulfate solution (Scharlau, Barcelona, Spain) at 10% were added. The mixture was filtered through filter paper, transferred to a separatory funnel, and 50 mL of Milli-Q ultrapure water at 8°C, followed by two extractions with 40 mL of chloroform (Sigma-Aldrich, Missouri, USA) were added. Chloroform was then collected in a separatory funnel and washed with 30 mL of Milli-Q ultrapure water at 5°C. Solvent was evaporated under vacuum at 35°C in a rotary evaporator (Quimis, São Paulo, Brazil), and the residue dissolved in 2 mL of methanol and filtered through anhydrous sodium sulphate (Sigma-Aldrich, Missouri, USA).

Standard solution preparation

AOH and AME were obtained from Sigma in crystallized form. A stock solution of 1,000 mg/L was prepared in methanol and kept at -20°C and a working solution (10 µg/mL) was also prepared in methanol. Calibration standards were prepared by dilution of the working solutions.

Mycotoxins analyses

Analysis were performed with a High-Performance Liquid Chromatograph coupled to a detector with a photodiode arrangement (DAD, Shimadzu, SPD-M10A DAD, Germany) and C18 reverse phase column; 10 µm average particle diameter, 3.9 mm internal diameter and 300 mm length (Waters, Ireland) with isocratic elution, using 80% HPLC grade methanol and 20% Milli Q ultrapure water as a mobile phase containing 300 mg of $ZnSO_4.7\ H_2O$/L at a flow rate of 0.7 ml/min. Detection was carried out in a wavelength of 250 nm. Volume injected was 20 µL and 30°C was the column temperature. The individual standard solutions were prepared at concentrations ranging from 200 to 500 mg/ mL in pure methanol. Stock solutions were stored at 20°C in the dark. For HPLC calibration, mixtures of working solutions were prepared (n=7) for the construction of calibration curves by serial dilution of the standard solution with methanol. Mycotoxins were quantified on a working range from 0.03 to 133 µg/mL for AME and 0.096 to 500 µg/mL for AOH by injection through seven points of the calibration curve (r=0.999).

Statistical analysis

The experiment was carried out using a completely randomized design, with 10 treatments arranged in a factorial scheme (3+1 tomato products x 3 brands) with 4 replicates (n=16 batches, 100 g each). Means were submitted to analysis of variance and were compared by the Tukey test (5%).

Results

Physico-chemical quality

Results found for physicochemical parameters (Table 1) revealed that only brand A tomato pulp is in disagreement with the current legislation, which establish the minimum content of 6% of natural soluble solids [20], significantly differing from other pulp brands.

Traditionally, soluble solids content characterize concentrated tomato products. Based on parameters established in Resolution CNNPA No. 12 of March 30, 1978 [21], the evaluated extracts are not in agreement with that resolution, as the minimum content of soluble solids for this product are classified as simple concentrate, discounting sodium chloride that should be at least 18%.

The same resolution establishes a minimum of 35% dry matter for ketchup. None of the evaluated brands is in agreement with this parameter, since they showed high moisture content and low total solids content (Table 1). For tomato pulp, there are no specific parameters for physicochemical quality established by law. Soluble solids content was higher in ketchup sachets followed by ketchup in bottle, extract and tomato paste.

When evaluating three different brands of tomato pulp and tomato paste Bery et al. [22] found soluble solids averaging 11.29 °Brix and

Sample/ Brand		Physicochemical characteristics[1]				
		Water content (g/100 g)	Total Solids (g/100 g)	Soluble Solids (ºBrix)	pH	Titratable Acidity
Ketchup	A	73.17 ± 0.54[a]	26.83 ± 0.54[a]	28.32 ± 0.16[a]	3.85 ± 0.03[a]	1.32 ± 0.01[a]
	B	69.04 ± 0.30[b]	30.96 ± 0.30[b]	32.47 ± 0.10[b]	3.80 ± 0.03[b]	1.51 ± 0.04[b]
	C	75.72 ± 0.48[c]	24.28 ± 0.48[c]	27.32 ± 0.12[c]	3.88 ± 0.04[c]	1.75 ± 0.07[c]
Paste	A	88.85 ± 0.39[a]	11.15 ± 0.39[a]	10.55 ± 0.17[a]	4.37 ± 0.02[a]	0.53 ± 0.01[a]
	B	87.84 ± 0.39[b]	12.16 ± 0.39[b]	12.80 ± 0.13[b]	4.34 ± 0.03[b]	0.70 ± 0.01[b]
	C	87.31 ± 0.80[b]	12.69 ± 0.80[b]	12.87 ± 0.12[a]	4.38 ± 0.01[a]	0.69 ± 0.01[b]
Pulp	A	93.68 ± 0.23[a]	6.27 ± 0.24[a]	5.86 ± 0.05[a]	4.06 ± 0.02[a]	0.13 ± 0.01[a]
	B	92.29 ± 0.21[b]	7.55 ± 0.19[b]	6.92 ± 0.09[b]	4.12 ± 0.02[b]	0.16 ± 0.01[b]
	C	90.82 ± 0.29[c]	8.89 ± 0.16[c]	8.00 ± 0.09[c]	4.18 ± 0.01[c]	0.20 ± 0.01[c]
Ketchup sachets	C	71.27 ± 0.15	28.73 ± 0.15	33.19 ± 0.16	4.01 ± 0.03	0.63 ± 0.03

[1] Data presented as mean ± standard deviation. Means followed by the same letter, in the same column, in a given product, have no significant differences at 5% probability by the Tukey test.
[2] Expressed as titratable acidity per g of acetic acid % (m/m) for ketchup and titratable acidity per g of citric acid% (m/m) for paste and pulp.

Table 1: Physicochemical analyses results of tomato products from the local market, Brasília-DF, Brazil.

Product	Brand	Foreign material (100 g)[1]	Micelian Filament Counting (Howard) %[2]
Ketchup	A	02 insect fragments	5.33 ± 1.33 (24.95)
	B	03 insect fragments	7.55 ± 2.04 (26.97)
	C	02 mites and 02 insect fragments	7.55 ± 2.77 (36.74)
Tomato pulp	A	04 rat hair and 01 mite	10.66 ± 4.81 (45.07)
	B	02 insect fragments	7.44 ± 1.65 (22.11)
	C	03 insect fragments and 01 whole insect	12.00 ± 4.62 (38.50)
Tomato paste	A	01 rat hair 02 insect fragment	7.99 ± 2.31 (28.89)
	B	absence	12.88 ± 2.04 (15.81)
	C	02 insect fragment	9.78 ± 2.04 (20.84)
Ketchup sachets	C	04 insect fragment	12.67 ± 0.94 (7.43)

[1]Results obtained by examining four aliquots from four samples of the same lot.
[2]Data presented as mean ± standard deviation (variation co-efficient in %) from four replicates by sample.

Table 2: Results of fungal counts by the Howard method and foreign material.

16.82 ° Brix, respectively, which are significantly lower than those found in this work. In a similar study, Silva et al. [23] found soluble solids ranging from 15.1 to 21.9 °Brix in commercial ketchups from Brazilian local market, which are also lower than those found in present study.

According to Jayathunge et al. [24], tomato paste must have pH ranging from 4.0 to 4.5. All evaluated extract brands are within this range. Bery et al. [22] found average pH of 4.15 and 4.13 for tomato pulp and paste, respectively, in agreement with the results found in this study. The pH range determined in different tomato products samples is associated with a low probability of microbial growth.

Bayod et al. [25] found low soluble solids content (26.2 to 27.1° Brix), total solids (27.16 to 27.43 g $100g^{-1}$) and similar pH values (3.8) in three ketchup brands in the Swedish market. Bannwart et al. [26] found values similar to those reported in this study for acidity (1.49 ± 0.15% acetic acid) when evaluating a conventional brand of ketchup obtained in the local market of Campinas-SP, Brazil.

Therefore, the absence of a standard established by law allows low soluble solids content products marketed, leading consumers to buy products with low quality parameters. Titratable acidity in tomato pulp is expressed in grams of citric acid per 100 g of the product, since this organic acid is predominant in raw material and commonly added as a preservative to tomato pulp [27].

Analysis of commercial products

Samples were analyzed for the presence of insect fragments, mites, rodent hair, and fungal counts carried out by the Howard method to assess the quality of raw material used in the preparation of tomato products (Table 2).

Only brand B tomato extract showed no foreign material. Insect fragments, rodent hair and mites were found in other samples. Rodent hair was found in tomato pulp and in tomato paste, and insect fragments were identified in all products of all brands, except brand B extract. Only brand C ketchup and brand A pulp had mites. Brazilian law determines absence of foreign material, parasites and larvae in tomato products [21] and limits the presence of rodent hair [28].

Flies play an important role in food contamination. They can carry microorganisms such as *Shigella, Salmonella, Escherichia coli*, *Campylobacter jejuni* and *Vibrio cholera*. The presence of animal hair may indicate contact with animals or products with excrement and/or urine of mammals, among them, rodents. Mites, if ingested, can trigger allergic reactions in susceptible individuals [29-31].

Brazilian law (RDC 14, 2014) [32] tolerates the presence of one microscopic fragment of rodent hair in 100 g of tomato products. The presence of rodent hair in the field is usual, particularly in spinach, peas and processing tomatoes. At least nine states in the USA reported this problem in the 80`s [33,34]. According to the Food and Drug Administration (FDA), the presence of rodent hair is an "aesthetic contaminant" (offensive to senses), since it entails no risk to consumer health, as tomato products are subjected to heat treatments (pasteurization and sterilization). There are no reports of individuals or animals who had leptospirosis or hantavirosis by eating processed food containing rodent hair [35].

Regarding mycelia filament counting by the Howard method, evaluated samples were adequate. Howard method has been used in the food industry for over half a century to determine the adoption of food

Product	Sample	AME (µg/g)*	AOH (µg/g)*
Ketchup Brand A	1	nd	nd
	2	nd	nd
	3	nd	nd
	4	nd	nd
Ketchup Brand B	1	nd	1.16
	2	nd	1.03
	3	nd	0.49
	4	nd	0.54
Ketchup Brand C	1	nd	nd
	2	nd	nd
	3	nd	nd
	4	nd	nd
Ketchup sachet Brand C	1	0.03	0.42

*nd: not detected. Data presented as mean of duplicates per sample.

Table 3: Occurrence of alternariol monometil ether (AME) and alternariol (AHO) in ketchup and in tomatoes infected by *A. alternata*.

safety procedures during processing. The low number of fungi found by this method does not ensure that the product was properly processed, but a high score always indicates deficiency in the selection process, with the use of rotten tomatoes. In tomato processing, decomposed material can appear in the final product if screening and selection of fresh fruits are not properly carried out. Fungi usually grow under tomato skin, yielding hyphae not removed by washing. Therefore, tomatoes with 4% rotted areas, may show 40% of positive fields, on the average, when evaluated by this method [36].

Processing tomatoes and their products may be contaminated with pathogenic microorganisms associated with the production environment: soil, irrigation water, organic fertilizers, water used in post-harvest process, and by the hands of workers handling fruits during harvesting and postharvest steps. Microbiological analyses of all samples did not show *Salmonella* sp in 25 g, coliform counting at 45°C less than 3 MPN/g, and coagulase-positive staphylococci less than 10 CFU/g. All products met Brazilian Legislation - Resolution RDC 12 (Brazil, 2001). Souza et al. [37] evaluated the quality of tomato paste produced in an industry of Goias, Brazil and obtained satisfactory results for microbiological quality. All tomato products were subjected to pasteurization (pH less than 4.5).

Occurrence of AME and AOH in tomato products

Mycotoxins were not identified either in tomato pulp or paste from the analyzed brands (Table 3). Mycotoxins were identified in one ketchup sample. The fungus associated with the identified mycotoxins was *A. alternata*. According to Bannwart et al. [26] ketchup is made from tomato pulp, in fresh form or in concentrated paste. From all concentrates, ketchup showed the highest content of solids, which could explain the presence of mycotoxin, once this tomato product is usually made of fully ripe tomatoes. In addition, several parameters determine ketchup characteristics such as the quality of raw material and processing conditions [38].

Stack et al. [39] evaluated 142 samples of ketchup from a production line and did not found AME, however TeA was identified in 73 samples at concentrations ranging from 0.4 to 70 µg/g. Terminiello et al. [6] evaluated 80 tomato pulp samples processed and marketed in Argentina and found 39 contaminated by *A. alternata* mycotoxins. TeA was found in 23 samples (39 to 4,021 µg/g), AOH in five (187 to 8,756 µg/g) and AME (84 to 1,734 µg/g) in 21 samples. The occurrence of two mycotoxins at the same samples was observed in 10 products.

Pavón et al. [40] evaluated 13 different samples of ketchup and found AOH in two, at concentrations of 460 and 680 µg/g, but in 20 tomato samples with symptoms of fungal infection, 11 showed high concentrations of AOH, ranging from 24,670 to 73,490 µg/g. The joint processing of healthy and contaminated fruits with *Alternaria* promotes reduction in mycotoxin content by dilution, but the process is unable to eliminate the mycotoxin from the final product. This might explain its identification only in infected tomatoes and ketchup, a concentrated product with high solids content.

Stinson et al. [41] evaluated the production of mycotoxins in *Alternaria sp.* contaminated apples and tomatoes species and found that from seven strains of *A. alternata*, six jointly produced AME and AOH mycotoxins in concentrations ranging from 0.35 to 4.35 µg/g and from 0.33 to 2.40 µg/g, respectively. For the fungus *A. alternata* 584S, authors found AOH mycotoxin production means in tomatoes (16.8 µg/g), similar to the present study (15.98 to 18.18 µg/g), suggesting that *Alternaria sp.* producing mycotoxins species such as *A. tenuis, A. alternata, A. tenuissima* and *A. solani* can produce abundant amounts of mycotoxins in infected fruits, in which the high moisture content can be the determining factor. However, authors emphasized that further studies would be necessary to establish whether mycotoxins were formed in intact fruit and found in processed products.

When assessing whole fruits of tomato, apple, orange and lemon, Stinson et al. [41] concluded that the main mycotoxin produced in tomato was tenuazonic acid, in concentrations higher than 13.9 µg/g, but reduced amounts of AME, AOH, and ALT were present, and the occurrence of AOH was higher than AME. AOH concentrations ranged from 0.3 to 5.3 µg/g in whole tomatoes contaminated with *Alternaria sp*. Although the authors unidentified the contaminant species, it is evident that the production of mycotoxins in naturally contaminated fruits is frequent and raises the possibility that infected tomatoes can be incorporated to processed products, in misclassification/selection or even by neglect, contributing to a potential risk to consumers health.

In assessing fresh tomatoes with apparent fungal lesions from the market in Denmark and Spain, Andersen and Frisvad [42] observed that the predominant genus *Alternaria* was present in 40% of the samples. Harwig et al. [43] also found *Alternaria* in 37% of tomatoes with fungal infection symptoms from Ontario, Canada, and Mislivec et al. [44] in 47% and 60% of tomatoes with mold in the eastern and Midwest regions of USA. In the State of California, the largest processing tomato grower in the USA, the occurrence of *Alternaria* was only 23%, whereas *Aspergillus* was identified in 57% of infected tomatoes. Muhammad et al. [45] also observed a high frequency of tomatoes infected with *Aspergillus* in Nigeria. Thus, the hypothesis that climatic and geographical factors may interfere with tomato microbiota was raised, and contamination by *Aspergillus* would be more common in dry and hot climates and *Alternaria* in humid and temperate ones [42].

AME and AOH were identified in tomato pulp and ketchup evaluated in 2004 and 2006 in the Czech Republic [46]. From a total of eight ketchup samples evaluated in 2004, AOH and AME were present in all, in concentrations of 6.9 µg/g and 1.6 µg/g, respectively. However, in the samples evaluated in 2006, AOH was identified in 17 out of 21, with values from 0.1 to 3.7 µg/g and AME in all 21 samples with values from 0.06 to 1.2 µg/g. The concentrations of AOH and AME reported by this author are lower than those found in this study (Table 3), which can be explained by the difference in the composition of the ketchups evaluated, quality of the raw material, climatic conditions and geography, as well as the fungi species infecting the raw material,

and the methodology used. As shown by Stinson et al. [41], species of the genus *Alternaria* produce varying amounts of mycotoxins AME, AOH, TeA and ALT on a same substrate; then, concentrations of these mycotoxins in tomato products also depend on the contaminating strain of the raw material.

Although there is a consensus that *Alternaria* genus is the main cause of fungal decay in tomatoes [46,47], studies concerning the occurrence of mycotoxins in tomatoes and tomato products are still scarce. However, there are reports of AME, AOH and TeA in wine [48], grape juice [49], barley, wheat and oats [50] and in peas [51].

In China, Li and Yoshizawa [52] identified *A. alternata* in 87.3% of wheat samples infected by fungi. They evaluated 22 wheat grain samples from the 1998 crop for the presence of *Alternaria* mycotoxins by high-performance liquid chromatography. AOH was detected in 20 of 22 samples at concentrations from 116 to 731 μg g^{-1} and AME at concentrations from 52 to 1,426 μg/g in 21 samples.

Noser et al. [53] evaluated 19 ketchup samples from the local market in Switzerland and identified AME and AOH mycotoxins in three products at concentrations from 4 to 5 μg/g and 1 μg/g, respectively. Regarding mycotoxin TeA, values varied from 3 to 141 μg/g, which led the authors to conclude that TeA was the prevalent mycotoxin in tomato products. Values found by these authors are lower than those reported in this study for mycotoxins. In Brazil, Motta and Soares [7] evaluated the occurrence of AOH, AME and TeA by liquid chromatography with diode detector in tomato products processed and marketed in Brazil. They tested eighty samples of tomato products. TeA was found in seven samples of tomato pulp at concentrations ranging from 39 to 111 μg/g, and in four samples of tomato paste (29 to 76 μg/g), but neither AME or AOH were detected.

High detection limits can explain how some methods do not get results for certain *Alternaria* mycotoxins and the fact that Motta and Soares [7] found no AOH and AME in their samples of tomato products. Although the detection limit has not been evaluated in this study, the identification of AOH and AME in sample of ketchup and tomato was possible.

In the literature, the highest levels of AOH were quantified in vegetables, nuts and oilseeds and in grains and products from cereals for animal feed. The lowest concentrations were found in cereals and processed products for human consumption, and in fruits and fruit products; but for AME, the highest levels were measured in grains and grain-based products used as feed, while the lowest concentrations were observed in fruit juices and vegetables, grains and products from cereals [54].

Alternaria mycotoxins are present in relatively high amounts in tomatoes, tomato products and other foods such as grapes, peas, wheat, apple, wine, carrots and corn [48-53,55]. However, there is no legislation establishing tolerances or limits for *Alternaria* mycotoxins in food. AOH, AME and TeA are not even among the worldwide most important micotoxins, such as aflatoxins, zealerona, ochratoxin, fumonisin, patulin, and trichothecenes [56]. The lack of tolerance limits for *Alternaria* mycotoxins in food can be justified on some points raised by the *European Food Safety Authority* (EFSA), such as the need for certified reference materials, definite performance criteria for food and feed analyses, the need for additional studies on the influence of the intake of these mycotoxins present in food (human and animal), besides the lack of toxicity data for AOH and AME that allows risk assessment [54].

Several authors [46,57-59] analyzed the natural occurrence of *Alternaria* sp. toxins in food. According to Ostry [46], the highest levels of *Alternaria* toxins were found in commercially available products (1 to 1,000 μg/g). The highest levels were found in food samples visibly infected with *Alternaria*, not suitable for human consumption.

EFSA [54] data showed that AOH was quantified in 3% of tomato samples and its products in concentrations ranging from 5 to 8,756 μg/g. In the present study, values higher than 8,756 μg/g were identified in tomato products (Table 3). AME was identified in 13% of samples available in the literature up to the year 2011 in concentrations from 0.2 to 1,734 μg g^{-1}. Terminiello et al. [6] reported the highest concentration of AME in a sample of tomato pulp (1,734 μg g^{-1}), whereas Motta and Soares [7] found no AME and AOH in tomato products. In Europe, AOH was identified in 13% of samples of lentil evaluated by Ostry et al. [49] and AME in 6% of linseed samples evaluated by Královál et al. [51].

Various techniques are adequate for quantification of *Alternaria sp.* toxin in food and feed. However, there are several limiting factors for the analysis of mycotoxins, such as extraction efficiency, availability of standards, and lack of reference materials for food and feed. Besides, most of the methods of analysis do not have interred laboratory validation studies, standardization of analytical methods or proficiency testing. In the literature, there are no test reports for risk assessment of the intake of *Alternaria sp.* toxins in food and feed at national or international levels. The lack of regulation on these mycotoxins in food and feed is a reality in Europe and other regions [54].

Conclusion

Tomato products evaluated showed differences in their physical and chemical characteristics, but are in accordance with the current legislation in Brazil. Regarding microbiological quality, all brands and products (paste, pulp and ketchup) are also in accordance with the legislation. Insect fragments, mites and rodent hair were identified in almost all brands and products, within acceptable limits. AOH and AME mycotoxins produced by *Alternaria alternata* were identified only in ketchups.

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Citation: Santos GG, Mattos LM, Moretti CL (2016) Quality and Occurrence of Mycotoxins in Tomato Products in the Brazilian Market. Enz Eng 5: 156. doi:10.4172/2329-6674.1000156