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Water activity and temperature effects on mycotoxin production by *Alternaria alternata* on a synthetic tomato medium

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ABSTRACT

Alternaria spp. have been reported to be the most frequent fungal species invading tomatoes. Certain species, in particular the most common one, *A. alternata*, are capable of producing several mycotoxins in infected plants and in agricultural commodities. Alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TA) are some of the main *Alternaria* mycotoxins that can be found as contaminants of food. The objective of this study was to determine the effect of water activity (a_w, 0.904, 0.922, 0.954, and 0.982) and temperature (6, 15, 21 and 35 °C) on mycotoxin production on a synthetic tomato medium of a cocktail inoculum of five strains of *A. alternata* isolated from tomato fruits affected by Blackmould. The optimum AOH production occurred at 0.954 a_w after 28 days of incubation at 21 °C. A temperature of 21 °C was the most favourable for AOH synthesis at all a_w levels. The maximum concentration of AME was determined at 0.954 a_w and 35 °C. The optimum conditions for TA accumulation were 0.982 a_w and 21 °C. At the 0.904 a_w no growth or germination was registered at 6 °C and 15 °C over the whole incubation period. At 21 °C and 35 °C growth occurred slowly but none of the toxins were detected at this a_w level. In general, high a_w levels were favourable for mycotoxin production. None of the other toxins was detected at quantifiable levels at 6 °C after the whole incubation period. A storage temperature of 6 °C or below could be considered as safe for tomato fruits and high moisture tomato products (a_w>0.95), in relation with *Alternaria* toxins. The results obtained here could be extrapolated to evaluate the risk of spoilage in tomato fruits and tomato products caused by this pathogen.

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1. Introduction

The genus *Alternaria* includes both plant-pathogenic and saprophytic species which may affect crops in the field or cause harvest and post harvest decay of plant products (Logrieco et al., 2009). As is common for many soft-skinned vegetables and fruits, tomatoes are especially susceptible to fungal invasion. *Alternaria* spp. have been reported to be the most frequent fungal species invading tomatoes (Barkai-Golan and Paster, 2008).

Species of *Alternaria* are known to produce many metabolites which play an important role in plant pathogenesis. Certain species produce several mycotoxins in the infected plants (Logrieco et al., 2009). *Alternaria* spp. can produce a wide variety of metabolites belonging to three different structural groups: (i) the dibenzopyrone derivatives, alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT); (ii) the perylene derivatives altertoxins (ATX-I and II); and (iii) the tetramic acid derivative, tenuazonic acid (TA). TA, AOH, AME and ATX-I are the main *Alternaria* mycotoxins that can be found as contaminants of food commodities. They are produced by *Alternaria alternata* which is considered the most common *Alternaria* species in harvested fruits and vegetables (Barkai-Golan and Paster, 2008).

The chemical and toxicological aspects of the *Alternaria* toxins have been recently reviewed (Logrieco et al., 2009; Ostry, 2008; Scott, 2001). Of particular health concern is the association found between *A. alternata* contamination in cereal grains and the high levels of human esophageal cancer in China (Liu et al., 1991). The toxicity of TA has been reported in plants, in chick embryos and several animal species, including guinea pigs, mice, rabbits, dogs, and rhesus monkeys (Solfrizzo et al., 2005). In dogs, it caused haemorrhages in several organs and in chickens sub-acute toxicity was observed. Precancerous changes were observed in esophageal mucosa of mice. The possible involvement of TA in the etiology of inlay, a human hematological disorder occurring in Africa, has been suggested (Scott, 2004). AME and AOH were mutagenic in microbial and mammalian cell systems (An et al., 1989; Liu et al., 1992; Scott and Stolz, 1980). There is also some evidence of carcinogenic properties: squamous cell carcinoma were induced in mice subcutaneously inoculated with

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human embryo esophageal tissue that had been treated with alternariol; and NIH/3 T3 cells transformed by AME were subcutaneously tumorigenic in mice (Scott, 2004). Lehmann et al. (2006) have recently reported on the estrogenic potential of AOH, its inhibitory effects on cell proliferation, and its genotoxic effect in cultured mammalian cells. Altertoxin I and related compounds may cause acute toxicity in mice, and were reported to be more potent mutagens than AOH and AME (Ostry, 2008).

Natural occurrence of AOH and AME has been reported in various fruits, including tomatoes, olives, mandarines, melons, peppers, apples and raspberries, and also in grains, sunflower seeds, oilseed rape and pecans. The occurrence of low levels of AOH in processed fruit products – apple juice, processed tomato products, grape juice, red wine, cranberry nectar, prune nectar and raspberry juice – is possibly of human health interest; in apple juice, prune nectar and tomato products, AME has also been detected (Scott, 2004; Terminiello et al., 2006). TA has been shown to occur in several *Alternaria* infected fruits and vegetables, and in other foodstuffs, such as grains and seeds (Azcarate et al., 2008; Scott, 2001). It has also been found in tomato products and spoiled tomatoes in Canada and the US (Scott and Kanhere, 1980; Stack et al., 1985), in Brazilian tomato products (da Motta and Valente Soares, 2001) and in Argentinean tomato puree (Terminiello et al., 2006). Few studies on the stability and reactions have been carried out on *Alternaria* mycotoxins although in common with other mycotoxins they are probably quite stable. A major proportion of the toxins survived the autoclaving of tomatoes in producing tomato paste. Alternariol, alternariol monomethyl ether, and altertoxin I were stable in fruit juices and wine over 20 days or at 80 °C after 20 min (Lawley, 2010).

Mycotoxin production depends on the fungal strain, the substrate on which it grows and the environmental growth conditions. The two most important environmental factors on the development of fungi and mycotoxin production are water activity (a_w) and temperature (Magan et al., 1984). *Alternaria* species grow best at room temperature but also are capable of growing at low temperatures. For this reason they are often involved in spoilage of fruit and vegetables during refrigerated transport and storage (Barkai-Golan and Paster, 2008). Knowledge of the influence of environmental factors on growth and mycotoxin formation can be an important aid in predicting mycotoxin contamination of food. Furthermore, the a_w and temperature limits for growth and mycotoxin production are sometimes markedly different (Magan et al., 1984). Growth of mycotoxigenic *Alternaria* species and its relation with these factors have been described in different substrates (Lacey, 1992; Pose et al., 2009). However, no studies have been carried out on mycotoxin production by *Alternaria* strains isolated from tomatoes or on tomato products. Such information is important in developing realistic forecasting systems for predicting risk of mycotoxin contamination of fruits or processed products.

Due to the high incidence of *Alternaria* and its mycotoxins in commodities and food products in Argentina, the objective of this work was to study the effects of a_w and temperature on the production of AOH, AME and TA by strains of *A. alternata* isolated from tomato fruits affected by Blackmould on a synthetic tomato medium.

2. Materials and methods

2.1. Fungal strains

The five single spore *A. alternata* isolates used in this study were obtained from ripe tomato fruits affected by Blackmould. The isolates were identified according to Simmons (2007). The colony and sporulation characteristics of representative cultures of *A. alternata* EGS 34.016 (Mycological Services, Crawfordsville, IN) were determined and compared with those of the tomatoes isolates in standard condition culture. Single germinating conidia were transferred to Petri dishes containing Potato–Carrot-Agar (PCA) and incubated for 7 days at 25 °C. Based on sporulation patterns and conidial morphology, the isolates used in this work were grouped as *A. alternata*. Their toxigenic profile was evaluated in autoclaved polished rice at 40% moisture after incubation in the dark at 25 °C for 21 days (Li et al., 2001). The five strains selected were capable of producing AOH, AME and TA. All of them are maintained in the culture collection of Universidad Nacional de Quilmes, Buenos Aires, Argentina and in the culture collection of the Istituto di Scienze delle Produzioni Alimentare (ITEM fungal culture collection) of the Consiglio Nazionale delle Ricerche, Bari, Italy. Cultures were stored on PDA slants at 4 °C.

2.2. Medium

Mycotoxin production was determined on tomato pulp agar (TPA) designed for this purpose (Pose et al., 2009). This medium contained 800 ml of pulp of fresh tomatoes, 200 ml of distilled water and 15 g of agar per litre (pH 4.39). The a_w of the medium was adjusted with glycerol 87% p.a. (Merck 4094) to 0.982; 0.954; 0.922 and 0.904 ± 0.003. Water activity was measured with a water activity meter (Aqualab CX-2, Decagon Devices Inc., USA).

2.3. Inoculation and incubation

The isolates were grown on water-agar (agar 2%) for 15 days at 25 °C to obtain heavily sporulated cultures (Larone, 2002). A cocktail inoculum was prepared with the five strains according to Hocking and Miscamble (1995). Spores of each strain were placed in an aqueous solution of 0.05% Tween 80 (Biopack) of a_w adjusted with glycerol, to avoid affecting the a_w of the culture medium. After homogenizing, the suspension was counted using a Neubauer chamber. Under these conditions the inoculum concentrations varied between 1.5 and 3×10^5 spores/ml. TPA plates were inoculated centrally with a 1 µl calibrated loop of spore suspension. Inoculated plates of the same a_w were placed in closed sterile polyethylene bags containing a vessel with adjusted glycerol–water solution to minimize water transfer from or to the medium. Control plates were prepared and measured at the end of the experiment in order to detect any significant deviation of the a_w, and no change in any tested plate was detected. Sets of each treatment were incubated at 6, 15, 21 and 35 °C. The amount of AOH, AME and TA produced in each plate after incubating for 7, 14, 21 and 28 days was determined. Each set of conditions ($a_w \times$ temperature $\times$ time) was run by triplicate.

2.4. Mycotoxin extraction

AOH, AME and TA were determined by a modification of the method described by da Motta and Valente Soares (2000a,b). A 20 g portion of tomato pulp agar cultures was weighed and transferred to blender cup with the aid of 60 ml methanol. It was blended at low speed for 3 min and transferred to a glass funnel fitted with a fluted filter paper. An additional 20 ml methanol was used for washing the residues left in the blender cup. The methanol aliquots were combined with 25 ml of a 10% ammonium sulphate solution. The mixture was filtered, transferred to another separating funnel, and 20 ml hexane was added. The mixture was gently shaken for 1 min and the hexane phase was discarded after separation. Twenty-five millilitres of water at 8 °C were added to the methanolic phase in order to avoid forming an emulsion. Two extractions with 20 ml chloroform were conducted. The chloroform extract was evaporated in a rotary evaporator at 35 °C. The residue was dissolved in 2 ml HPLC grade methanol and was analyzed for AOH and AME. The methanolic phase was acidified to pH 2 with HCl (c). Two extractions with 20 ml chloroform were made. All the chloroform was collected in a separating funnel and washed with 15 ml water. The chloroform extracts were collected, filtered through anhydrous sodium sulphate, and evaporated in a rotary evaporator at 35 °C. The residue was dissolved in 4 ml HPLC grade methanol and was analyzed for TA.

2.5. Mycotoxin analysis

Mycotoxin analysis was performed by the method described by da Motta and Valente Soares (2001) with modifications. Standards and extracts were injected into an HPLC system consisting of a Shimadzu LC-CA liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a Rheodyne sample valve fitted with a 20 µl loop and a Shimadzu UV detector Model SPD-6A. The analytical column was Jupiter 4.6×250 mm 5 µ C18 (Phenomenex, USA). The mobile phase was methanol/water (80:20) containing 300 mg $ZnSO_4.H_2O$/l, for AOH and AME, and methanol/water (90:10) containing 300 mg $ZnSO_4.H_2O$/l, for TA. A flow rate of 0.4 ml/min was used. The wavelength for recording chromatograms was 258 nm for AOH and AME, and 280 nm for TA. A calibration curve was constructed for quantification purposes using the toxin standards and correlating peak area versus concentration (levels 100 to 1000 µg/kg). Confirmation of all toxins was achieved by co-injection with the corresponding standard and by using a Shimadzu SPD-M10Avp photodiode array detector. The spectra were acquired in the range of 200 to 400 nm on the apex and on the ascending or descending part of each peak, using a pilot signal at 258 nm for AOH and AME, and at 280 nm for TA. Reference spectra were acquired during the elution of associated standards and used for peak identification by comparison after spectra normalization. Detection limits of the method were established as the minimum concentration of the toxins detected in the samples that allowed confirmation by the diode array detector (2 µg/kg, 1.6 µg/kg and 2.5 µg/kg for AOH, AME and TA respectively). Noise was calculated as six times the standard deviation of the lineal regression of the drift at the selected time range. The quantification limits of the toxins were determined as five times the noise, and were 5 µg/kg for AOH, 3 µg/kg for AME, and 8 µg/kg for TA. Average recoveries for four levels of standard addition (100, 250, 500, and 1000 µg/kg) from triplicate spiked tomato pulp agar plates were 86%, 92% and 88% for AOH, AME and TA with coefficients of variation (RSDs) of 4.52, 5.52 and 6.37% respectively.

2.6. Statistical analysis

The effects of a_w and temperature on AOH, AME and TA accumulation at 7, 14, 21 and 28 days by *A. alternata* were analyzed statistically by ANOVA using Statistica software v6.0 (StatSoft, Inc., 1984–2001, Tulsa, OK, USA). Means were compared by LSD test to determine significant differences between the treatments assayed.

3. Results

The effects of a_w, temperature and incubation time on mycotoxin production by *A. alternata* strains on a synthetic tomato medium is shown in Figs. 1–3.

The ANOVA of the effect of a_w, temperature, incubation time, and their interactions showed that all factors alone and all the interactions were statistically significant in relation to AOH production ($p<0.0001$), and to AME and TA production ($p<0.05$).

At the lowest a_w level evaluated (0.904) no growth or germination was registered at 6 °C and 15 °C over the whole incubation period. At 21 °C and 35 °C growth occurred slowly but none of the toxins were detected at this a_w level.

3.1. Effect of a_w, temperature and incubation time on AOH production

The optimum AOH production occurred at 0.954 a_w after 28 days of incubation at 21 °C (143.7 µg/g) (Fig. 1). This a_w level was the most favourable for the synthesis of AOH on tomato medium. An increment of a_w to 0.982 resulted in a reduction of the amount of AOH produced at all temperatures assayed. At 0.922 a_w, AOH production was significantly inhibited; the highest concentration detected at this a_w was 6.2 µg/g

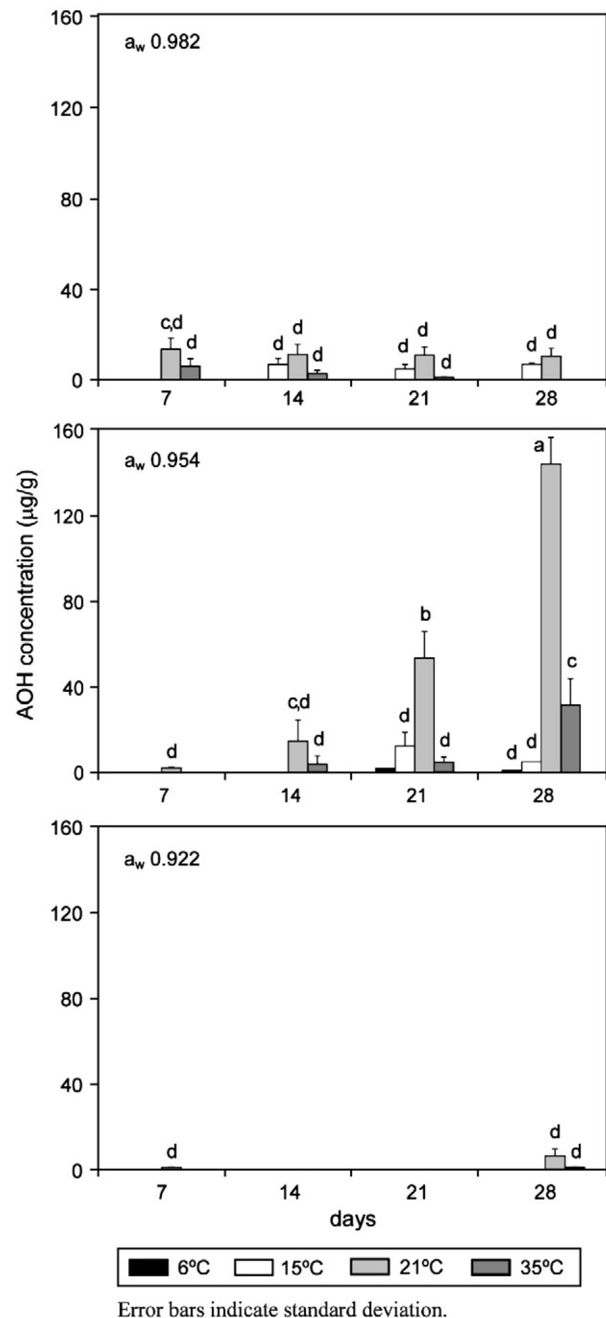

Fig. 1. Effect of water activity (a_w), temperature and incubation time on AOH production by *Alternaria alternata* strains on tomato pulp agar.

after 28 days of incubation at 21 °C. A temperature of 21 °C was the most favourable for AOH synthesis at all a_w levels assayed and during the whole incubation period. At 0.954 a_w, a variation from 21 °C to 35 °C produced a 78% reduction in the accumulation of AOH after 28 days. At 15 °C low amounts of AOH were detected even at the optimum a_w. At the lowest temperature (6 °C), the AOH production was completely inhibited at all a_w levels assayed.

3.2. Effect of a_w, temperature and incubation time on AME production

The effect of a_w on the synthesis of AME was similar to that observed for AOH (Fig. 2). The optimum a_w level for this mycotoxin was also 0.954. AME concentration decreased significantly with a variation of a_w at all temperatures evaluated. The maximum

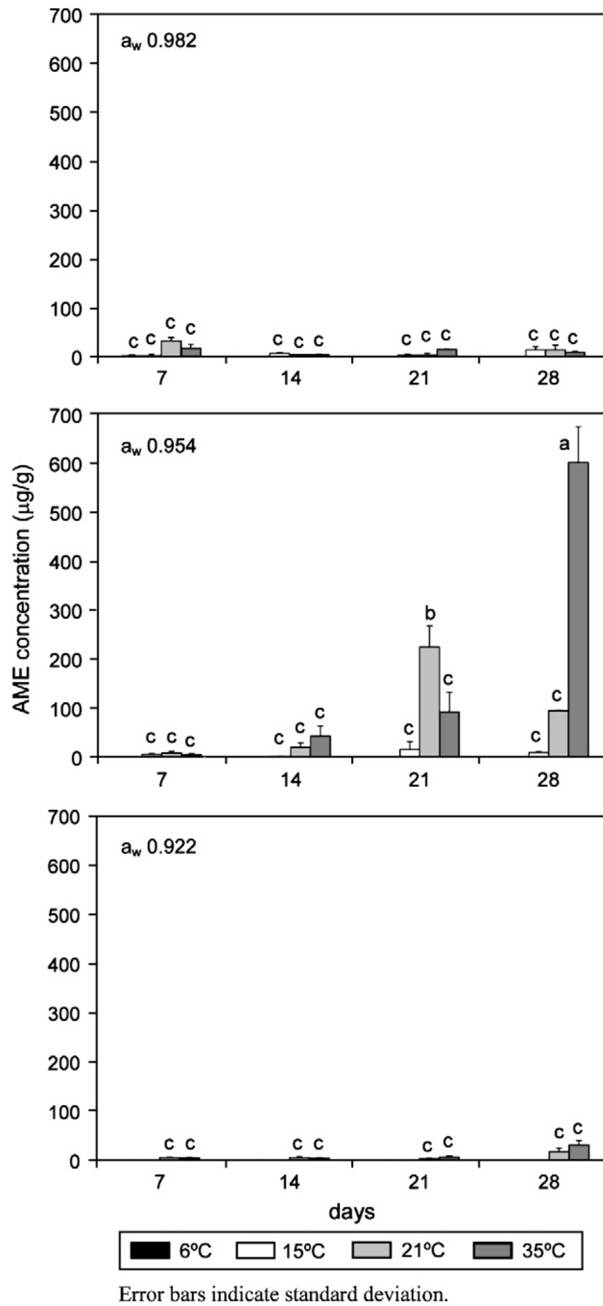

Fig. 2. Effect of water activity (a_w), temperature and incubation time on AME production by *Alternaria alternata* strains on tomato pulp agar.

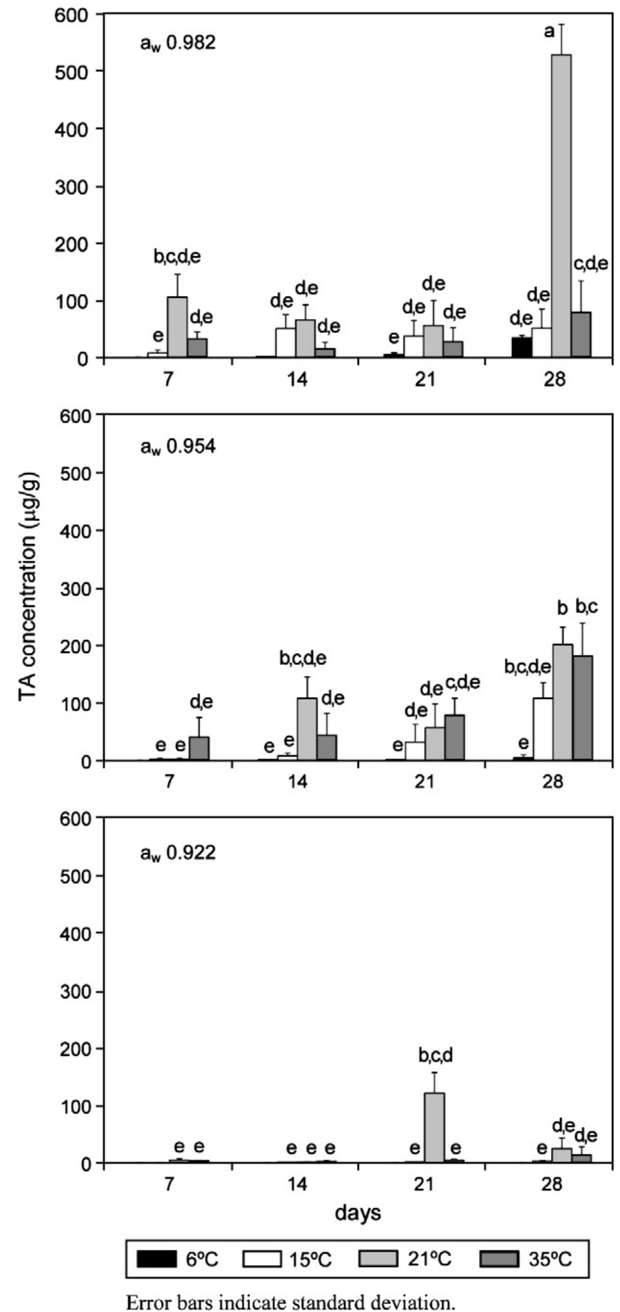

Fig. 3. Effect of water activity (a_w), temperature and incubation time on TA production by *Alternaria alternata* strains on tomato pulp agar.

concentration (600.9 µg/g) was determined at 0.954 a_w, 35 °C, and 28 days. At the other a_w levels, the amount of AME produced was very low during the whole incubation period. The maximum concentration of AME detected at 0.922 a_w was 30.2 µg/g (35 °C and 28 days of incubation), while at 0.982 a_w was 31.6 µg/g (21 °C and 7 days of incubation). The relationship between AME biosynthesis and temperature was different from that described for AOH. At the optimum a_w level (0.954), the highest concentration was detected at 35 °C and 28 days. However, at 21 days, the production was significantly higher at 21 °C than at 35 °C (223.6 and 91.7 µg/g respectively). At 15 °C AME concentration was extremely low at all a_w levels over the entire incubation period, with a maximum of 15.0 µg/g after 21 days at the optimum a_w level (0.954). At the lowest temperature assayed (6 °C) the production was inhibited; only traces were detected at 0.982 a_w at 7 days.

3.3. Effect of a_w, temperature and incubation time on TA production

The influence of a_w on TA production was different from that observed for AOH and AME (Fig. 3). In general, TA concentration increased with a_w. The maximum accumulation was registered at 0.982 a_w and 21 °C after 28 days (528.2 µg/g). At the same temperature and incubation time, a change to 0.954 a_w resulted in a 62% reduction on TA concentration. At 0.922 a_w the production of TA decreased at all temperatures, and only at 21 °C significant amounts were detected. The optimum temperature for TA synthesis was 21 °C at all a_w levels assayed. At the optimum a_w (0.982) and 28 days, the effect of temperature was stronger; a change from 21 °C to 35 °C or 15 °C resulted in an 85% and 90% reduction on the amount of TA detected respectively. At 6 °C, significant levels of TA were only detected at 0.982 and 28 days (33.9 µg/g).

3.4. Total mycotoxin accumulation after 28 days

The effects of a_w and temperature on the total mycotoxin accumulation after 28 days of incubation on a tomato medium are summarized in Table 1. A high accumulation of all three mycotoxins occurred at 35 °C and 0.954 a_w, although significant amounts were also observed at 21 °C and the same a_w level. The maximum TA accumulation was observed at 21 °C and 0.982 a_w; however, low levels of the other toxins were produced at this a_w–temperature combination. High a_w levels were favourable for mycotoxin production, although the optimum a_w value for AOH and AME differed from that for TA. A water activity below 0.92 would be inhibitory for production of all tested mycotoxins by *A. alternata* on tomato medium.

At temperatures between 21 °C and 35 °C high quantities of *Alternaria* mycotoxins were produced; however, at 15 °C the concentration of all three toxins decreased significantly. At 6 °C only small amounts of TA were detected the highest a_w level (0.982) after 28 days of incubation. None of the other toxins was detected at quantifiable levels at this temperature after the whole incubation period.

4. Discussion

Comparison with literature data is difficult since there is little information on the combined effect of a_w or temperature on mycotoxin production by *A. alternata* on tomato or tomato media, and very few reports on other substrates.

Ozcelik et al. (1990) inoculated an *A. alternata* strain into tomatoes and found that concentrations of up to 120.6 mg of AOH and 63.7 mg of AME per 100 g of tissue were produced in tomatoes stored at 15 °C for 4 weeks. These values are much higher than those detected in the present work at the same conditions. These authors have also detected AOH and AME in tomatoes stored at 4 °C; however, no TA was detected in tomatoes stored at 4, 15 or 25 °C for up to 5 weeks. Hasan (1995) reported that 28 °C was the optimum temperature for AOH and AME production by *A. alternata* on tomato homogenate cultures; but TA was produced in large quantities at 21 °C. All toxins were produced in reasonable amounts at 7 °C on tomato homogenate.

Few studies have determined the optimal conditions responsible for mycotoxins production in *Alternaria* spp. Sanchis and Magan (2004) have reported that the limiting a_w for *A. alternata* to produce mycotoxins (ALT, AME and AOH) on wheat grain is 0.88–0.89 a_w. Optimum production of the toxins was at about 25 °C and a_w greater than 0.97 (Sanchis and Magan, 2004). Magan et al. (1984) reported that at 0.98 a_w and 25 °C, a single colony of *A. alternata* grown on wheat extract agar produced 807 μg of AOH and 603 μg of AME per ml in 30 days. However, production of both mycotoxins at 0.95 a_w, was less than 40% of these amounts. Both metabolites were produced at 5 °C and 0.98 to 0.95 a_w, and at 30 °C and 0.98 to 0.90 a_w. Hasan (1996) found that the optimum temperature for toxin production in synthetic media by *A. alternata* IMI 89344 was 28 °C for AOH and AME, and 21 °C for TA. Some ecological studies have been carried out on tenuazonic acid (TA) production by *A. alternata* and *A. tenuissima* species on sorghum and cottonseed (Magan and Baxter, 1994; Young et al., 1980). Minimum water availability conditions for production were found to be about 0.93–0.90 a_w in vitro on sorghum-based media.

Differences observed between literature data could be attributed to substrates, strain variability, geographic differences between isolates, etc. In general, a limiting a_w value for mycotoxin production by *A. alternata* could be concluded to be in the range of 0.88–0.90 a_w.

The limiting temperature for mycotoxin production determined by different authors is more variable, since there are reports of mycotoxin production at temperatures as low as 4 °C. According to our results, a storage temperature of 6 °C or below could be considered as safe for tomato fruits and high moisture tomato products ($a_w > 0.95$), in relation with *Alternaria* toxins. These results are in agreement with other literature data. Hasan (1995) reported that to control the growth and toxin production in tomato fruits, the temperature should be maintained below 7 °C, and the storage period should not exceed 10 days. Even though the biosynthesis of AOH and AME are affected differently than TA by environmental factors, a low storage temperature would be effective in controlling production of all three toxins in tomato products.

Since *Alternaria* species are ubiquitous in nature and are very commonly occurring postharvest pathogens of tomatoes, it is not surprising that *Alternaria* mycotoxins are found in tomato products. A synthetic medium (TPA) emulating the composition of tomato fruits was developed in a previous work to determine the influence of a_w and temperature on germination and growth of *A. alternata.* The use of TPA provided a practical advantage, as it is easier to adjust the a_w value in a synthetic medium, and it showed good performance in the evaluation of mycotoxin production as a function of environmental parameters.

The concept of using cocktail inocula was introduced for physiological studies on foodborne bacterial pathogens, particularly in acquisition of data for predictive modelling studies, as a way of determining the extremes of growth limits for particular species. The use of bulked spore suspensions was applied for the first time to studies on the a_w tolerances of fungi by Hocking and Miscamble (1995). Although this approach can be criticized because of loss of information about the responses of individual strains of a species, it is accepted as a legitimate method for establishing the most extreme conditions under which a particular species is capable of growth. The use of a cocktail inoculum of regional strains isolated from tomato fruits affected by Blackmould in the present study provided data for the determination of limiting conditions at which mycotoxins can be produced on tomato products.

The a_w range studied was selected considering the optimum and minimum a_w values reported for mycotoxin production by *A. alternata* on different substrates. Intermediate values were chosen according to a_w values of tomato products (tomato sauce: 0.986; tomato chutney: 0.955; concentrated tomato paste: 0.93–0.85). Although *Alternaria* is probably inactivated during thermal processes, the raw material used for elaboration of tomato products (tomato paste) is prone to contamination if it is not stored at adequate temperatures before processing. At this stage mycotoxin accumulation could increase. According to our results, concentrated tomato pastes, which are often used in the production of tomato sauces and purees, are also susceptible to contamination in spite of their low a_w (0.93 a_w). Tomato products of a_w lower than 0.92 present low risk of contamination with *Alternaria* toxins. Most industrialized tomato products generally have a_w values higher than 0.92, with the exception of some concentrated tomato

Table 1
Effect of a_w and temperature on mycotoxin production by *A. alternata* strains on a synthetic tomato medium after 28 days of incubation.

Temp (°C)	a_w	Mycotoxin production (μg/g)[a]		
		AOH	AME	TA
6	0.982	n.d.	n.d.	33.9 ± 4.2
	0.954	Traces	n.d.	Traces
	0.922	n.d.	n.d.	n.d.
15	0.982	7.0 ± 0.4	13.2 ± 5.3	50.3 ± 13.5
	0.954	5.0 ± 0.1	8.9 ± 1.2	107.5 ± 27.3
	0.922	n.d.	n.d.	Traces
21	0.982	10.7 ± 2.5	13.2 ± 2.6	528.2 ± 53.0
	0.954	143.7 ± 12.4	94.3 ± 0.9	200.8 ± 30.0
	0.922	6.2 ± 1.4	16.8 ± 4.1	24.8 ± 7.8
35	0.982	n.d.	8.0 ± 1.8	79.2 ± 18.5
	0.954	31.6 ± 6.7	600.9 ± 73.0	181.0 ± 36.9
	0.922	Traces	30.2 ± 6.6	13.5 ± 4.4

n.d.: Not detected.
[a] Mean and standard deviation from three independent replicates.

paste (0.93–0.85 a_w) and dried tomatoes (0.93–0.77). However, growth of *Alternaria* and mycotoxin production during the early stages of drying of tomatoes is also of concern, especially in high temperature climates.

Temperature is one of the major environmental factors that affect the shelf life of tomato fruits and their rate of deterioration by *A. alternata*. The biosynthesis of different mycotoxins produced by *Alternaria* spp. is favoured by different temperatures. The range of temperatures selected for the present study is representative of ambient temperatures at which tomato fruits are stored in warm temperate regions such as our country in the different seasons (35 °C, 21 °C, and 15 °C, in summer, autumn and winter respectively); and 6 °C was selected as a refrigeration temperature. Our results showed that warm storage temperatures increase the risk of contamination with *Alternaria* mycotoxins. Despite the diversity of data in the literature, we could conclude that refrigeration of these products would be advised in order to reduce the risk of toxin production by *A. alternata*.

5. Conclusions

The present study is the first report on the effect of a_w and temperature on mycotoxin production by *A. alternata* on tomato medium. The results obtained here could be extrapolated to evaluate the risk of spoilage in tomato fruits and tomato products caused by this pathogen. This knowledge is necessary to elaborate strategies combining different methodologies and controlled environmental factors to prevent tomato and tomato products contamination. The data presented here provide a matrix of the responses of three *Alternaria* mycotoxins to water activity and temperature that may be used in constructing a mathematical model for the prediction of health risk in these substrates. Such a model will be of benefit to manufacturers of these and related products in development of new formulations and processes.

Direct consumption of moldy tomatoes by the consumer is unlikely, but the possibility of moldy tomatoes being included in processed tomato products is much more likely (Andersen and Frisvad, 2004). Thus, the presence of *Alternaria* mycotoxins in tomato products is frequent, and sometimes they are found in high levels. Information on the influence of environmental factors on mycotoxin production is useful so that suitable storage environments can reduce the risk of contamination throughout the whole process.

There are no specific regulations for any of the *Alternaria* toxins in foods. However, considering toxicity studies and the frequency of their presence in tomato products, their analysis is necessary to evaluate the consumer health risk. Moreover, the presence of *Alternaria* toxins in tomato products might be indicative of the use of decaying tomatoes by the processing plants. The quantification of these mycotoxins in tomato products may be considered an indicator of the quality of raw material.

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