SCIENTIFIC PAPER

THE RELATIONSHIP BETWEEN ERGOSTEROL AND *ALTERNARIA* MYCOTOXINS IN TOMATOES WITH DIFFERENT SURFACE DECAYED PROPORTIONS

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Abstract

The aim of this study is to evaluate the relationships between the concentrations of ergosterol (ERG) and *Alternaria* mycotoxins (alternariol (AOH), alternariol monomethyl-ether (AME) and tenuazonic acid (TenA)) concentrations in tomato pulp and pomace samples, with different proportions (%) of decay in tomato surface. To evaluate such relationships is used a range of 89 to 99 percent of decayed tomato surface. For this study, is used the Rio Grande variety, one of Turkey's most common industrial type tomato varieties, for tomato paste production. Tomatoes were classified in percentage as 25, 50, 75, and 100 % rotting rate, based on visibly rotten by molds on their surface, before processing to tomato pulp. Some quality parameters of tomato pulp and pomace samples determined were: the soluble solids by refractive index measurements, pH, titratable acidity with the titrimetric method, and expressed as citric acid g/L, the color as reflectance with a chroma-meter, for three color components L*, a*, b*. To determine ERG, TenA, AOH, and AME in the samples was used the HPLC method, and for the proposed method are provided data on the linearity of the standard curve, limit of detection, recovery, and precision. Also, the correlations and significance levels between the tomato surface decay proportions, and the ergosterol and the *Alternaria* mycotoxins concentrations are evaluated, to examine the relationships status between variables, and to determine how the other variable explains many variations within one variable. The results revealed that the concentrations of ergosterol and *Alternaria* mycotoxins in tomato pulp and pomace were significantly ($p<0.05$) affected by different proportions of decayed tomatoes surface. The high correlations that exist among evaluated parameters, suggest strongly that such measurements are very important to determine the quality of tomato. Moreover, such evaluations could be considered important indicators to tomato processing industry, concerned to the quality defects. The results of this study may serve as new data for future follow-up studies in this field.

Keywords: *alternariol, alternariol monomethyl ether, decay, ergosterol, tenuazonic acid, tomatoes*

Highlights

- Ergosterol has the potential to be used as a quality parameter instead of *Alternaria* mycotoxins.
- Correlation between ergosterol and *Alternaria* of TPU samples with different TSD proportions.
- Depending on mold genera, of *Alternaria* and ergosterol can be produced in TPO and TPU samples.

INTRODUCTION

The presence of mold in processed foods is a clear indicator of non-ideal processing conditions and the use of raw materials that do not meet the required quality standards [1]. Fungi are known as organisms that produce many different secondary metabolites, and many of these metabolites are called mycotoxins. Mycotoxins play an important role in the pathogenicity of fungi and pose a serious threat to agricultural products [2]. However, many of these mycotoxins are known to have adverse effects on both animals and humans when consumed. Therefore, it is important to prevent unhealthy processing conditions and the use of low-quality raw materials in the food industry. Compliance with hygienic standards and quality controls during food production and processing can help prevent mycotoxins from entering the food chain [3, 4, 5].

Alternaria mold species are widespread, in both semi-arid and humid lands. *Alternaria* mold has the ability to spread, both to adapt to environmental conditions and to easily infect plant hosts [3, 6-8]. Many plants, including cereals, oilseeds, tomatoes, and citrus fruits, are susceptible to severe diseases caused by these fungi [9, 10]. Tomatoes are easily be infected by *Alternaria* species during and after harvest. As a result of these infections, black rot occurs on the fruits. This infection caused by *Alternaria* mold species causes significant problems in tomato production. Control and management of these pathogens are of great importance for tomato production and tomato products [11, 12].

Alternaria mold species are common fungal genera that, under appropriate conditions produce several metabolites, such as mycotoxins. One of the most critical mycotoxins producing species is *Alternaria alternata*. Researchers have stated that fruits and vegetables with soft peels, such as tomatoes are susceptible to the *A. alternata* invasion [13]. During ripening phase, water can be found on the surface of the fruit from the dew, or excessive irrigation. Fungus spores sprouting, which can be found on the surface of the fruit, are due to the accumulation of water [14].

Contamination of food with *Alternaria* mycotoxins occurs as a result of *Alternaria* species infecting food crops and agricultural products. *Alternaria* fungi can cause lesions on plant leaves, stems, or fruit. Apart from this, *Alternaria* toxins are formed in humid and hot environments during the storage of agricultural products. Additionally, it can also pass into food when agricultural products are processed. Mycotoxins are released into food during

processing of infected tomatoes, especially in processed foods such as tomatoes or tomato products. It has been reported in studies that *Alternaria* mycotoxins were detected in grains and products produced from grains [15, 16], feeds [17], milk and dairy products [18], nuts [19], oilseeds [20], vegetable oils [21] and fermented beverages [22].

In order to penetrate and grow in tomatoes, *A. alternata* needs a damaged or soft texture. Thus, during harvesting, fungus can penetrate the tomato peel, caused by physiological disorders (spotty ripening, cracking, sunburn, yellow spot), injuries by insects, puncture injuries, or the calyxs mold growth associated with the scar [23]. For many *Alternaria* spp. that could contaminate tomatoes, the biggest concerns arise from *Alternaria* mycotoxins, which are produced by such species [9]. Several *Alternaria* mycotoxins that are detected in moldy tomatoes include alternariol monomethyl ether (AME), alternariol (AOH), tenuazonic acid (TenA), altertoxin (ATX), and altenuenes (ALT) [9], found even in tomato products with potentially adverse effects on human health [6-8]. The presence of TenA in tomato products may indicate that rotten tomatoes were used in the processing procedure [6]. So, to prevent mycotoxins contamination, damaged or mold-decayed tomatoes should not be used in the processing of tomato products [25].

Even in our daily consumption of fruits and vegetables, these mycotoxins may be considered toxic contaminants, as *Alternaria* mycotoxins are able to be produced naturally in them in case of infection [3]. Even in Europen Food Safety Authority's (EFSA) opinion [8], it stated the need for defined performance criteria and certified reference materials for the analysis of *Alternaria* toxins in various foods and feeds. EFSA has been collecting information on *Alternaria* toxins in food and processed products for a long time. According to EFSA, tomatoes and tomato products are also exposed to mycotoxins [26]. The co-occurrence of *Alternaria* toxins and metabolites in foods constitutes a problem and remain to be investigated, as well stated in the International Regulations of Commercial Products [3]. This situation causes a number of difficulties in the trade of products coming from different geographies around the world as a part of international trade. Therefore, further investigation and control of the presence of these mycotoxins in foods is of critical importance both to protect consumer health and to make the food industry safer [27].

Ergosterol (ERG) (3β-hydroxy-5,7,22-ergostatriene) is a substance that forms the fungal cell wall and is widely found in all foods, especially tomatoes [28]. Ergosterol is a critical fungal sterol, the primary sterol in fungal membranes, and has a regulatory role in the

selective permeability and location in the membrane. It is considered an essential component of a healthy fungal cell. Studies have shown that there is a positive correlation between mold growth, ERG levels and subsequent toxin production. It is why the ERG has recently been recognized as an indicator for the determination of fungal growth. The detection of ERG presence is considered a significant quality parameter for the decision on the fungal growth levels in food [29-31]. Ergosterol is a part of mold membranes and is found in high levels in molds. In addition, it is a minor component of various plant and animal sterols. The sterol content in bacteria is insignificant. Because they constitute less than 0.01% of the dry weight and very little of it belongs to ergosterol. Ergosterol can also be found in the structure of yeasts, but molds are considered the primary producers due to their greater biosynthetic capacity compared to yeasts [32].

The presence of ergosterol is associated with the presence of molds in foods, but no specific conditions for ergosterol formation have been identified. However, it may be associated with conditions that cause mold growth. Some mold species, such as *Fusarium, Alternaria*, *Aspergillus, Penicillum*, and *Mucor*, have been reported to produce significant amounts of ergosterol [28]. Moreover, the presence of ergosterol is mainly associated with active biomass and not with total biomass. Marin et al. [33] also noticed the high correlation between ergosterol and colony diameter and emphasized the potential for use of both parameters. *A. flavus*, *B. fulva, B. nivea, F. oxsporum, M. plumbeus, P. commune* and *P. roqueforti* with the same colony diameter have also been reported to produce variable levels of ergosterol [34].

For identifying molds, the chemical and biochemical methods are based on the detection and quantification of some specific components, such as ERG, chitin, fungal volatiles, and adenosine triphosphate (ATP). For ERG analysis, the methods are based on direct saponification, hexane extraction, and quantification using TLC (Thin Layer Chromatography), LC (Liquid Chromatography) , HPLC (High Performance Liquid Chromatography), or spectrophotometric methods (UV, infrared) [8, 29-31, 35-39]. There exists a relationship between howard mold count (HMC)-ERG–mycotoxins depending on the decay proportion of figs, apples, nuts, and tomatoes [28], and depending on this relationship, ERG may be used as a microbiological fungal growth indicator. For this reason, various research studies are needed to examine the ERG-mycotoxin relationships in other foodstuffs and, to use ERG as an alternative to mycotoxin analysis as a microbiological quality parameter.

To the best of our knowledge, there is no published research study on the determination of the relationship between ERG and *Alternaria* mycotoxins in pulp and tomato paste obtained from tomatoes with different surface decay rates. The data obtained through this study can be considered an important indicator for the tomato processing industry in terms of product quality.

The objectives of this study are as follows;

1. To determine the contents of ERG and *Alternaria* mycotoxins (AOH, AME, TA) in tomato pulp (TPU) and tomato paste (TPO) samples obtained from tomatoes with different surface rot (25, 50, 75, and 100%).
2. To reveal the correlation between Ergosterol and *Alternaria* in TPU samples with different TSD ratios.
3. To investigate the potential of using ergosterol as a quality parameter instead of *Alternaria* mycotoxins.

MATERIALS AND METHODS

Tomato sampling and procedures

For this study, the Rio Grande variety, one of Turkey's most common industrial type tomato varieties, for tomato paste production, which is obtained from the Honaz Tomato Paste Factory, located in Honaz district of Denizli province, Turkey. This factory has a daily capacity of 50 tons of tomato processing. The samples were taken from eight different batch productions. With an average value of 4 kg of tomatoes taken for each decay proportion from each production batch. Samples were immediately transported to the university laboratory and processed further into tomato pulp (TPU). Tomatoes were classified in percentage as having a 25, 50, 75, and 100% rotting rate, based on visibly rotten by molds on their surface, before processing to TPU. Tomato surface decay (TSD) proportions were determined according to the method of Kadakal et al. [40].

The tomatoes were processed with a plant finisher (pilot-type Langsenkamp mfr., model 185S, Indianapolis, IN). Then, the inactivation of the pectolytic enzyme was achieved by heat treatment at 90°C for 3 minutes, and then cooled to 25°C in a cooling water circulating container [40]. After cooling, the samples were passed through a finisher with a series of three sieves, respectively equipped with 1.8, 0.71, and 0.5 mm mesh size. Separated pomace and pulp tomatoes samples using a finisher, and were kept at -20°C until the final determinations.

Determination of some quality parameters: soluble solids, pH, titratable acidity, and color measurement

The soluble solids of TPU samples were determined by refractive index measurements as the mean of two parallels and expressed as Brix degree (ºBx) using a digital refractometer, model RFM 340 (Bellingham & Stanley Co., Atlanta, GA) [40-42].

The pH of TPU samples was measured with a pH meter, model WTW pH 537 (WTW Measurement System Inc., Fort Myers, FL). The titratable acidity (TA) of TPU samples was measured with the titrimetric method, and expressed as citric acid g/L. The results are expressed as the mean of two parallel measurements [40, 41, 43].

The color of the TPU samples is measured as reflectance with a chroma meter, model Konica Minolta CR-300 (Minolta Co., Osaka, Japan). As color values are measured for three color components (Hunter L*, a*, b*). The food industry has widely adopted this effective system, Hunter L* a* b* color space, for measuring color differences. On the tristimulus coordinate system, the L* value indicates lightness (0 is black and 100 is white), the a* value indicates red (+), or green (-) color, and the b* value indicates yellow (+), or blue (-) color [44]. Results are calculated as the mean value of five parallel measurements.

Determination of Ergosterol and *Alternaria* mycotoxins

The HPLC method was used to determine ERG in the samples according to Kadakal et al. [10]. ERG an analytical-grade reagent, in crystalline form, was obtained from Sigma (Sigma-Aldrich Chemie GmbH, Deisenhofen-Germany). To determine TenA, AOH, AME was used the HPLC method, according to Terminiello et al. [45]. Each of the standards for AOH (*Alternaria sp.),* AME (*Alternaria alternata* (*tenuis*), and TenA (*Alternaria alternata*) were obtained from Sigma (St. Louis MO, USA).

The chromatographic separation was performed with an Inertsil 100A ODS-3 reversed-phase column (4.6×150 mm, 5 μm BGB Analytik AG, Boeckten, Switzerland). A photodiode array detector (SPD-M10 Avp, Shimadzu, Kyoto, Japan) set at 282 nm, a Shimadzu LC-10AT-VP HPLC pump, and a column oven (CTO-10AS, Shimadzu) set at 25°C. The sample (20 μl) was injected into the HPLC system with a syringe (Hamilton Co., Reno, NV). The LabSolutions software (Shimadzu) was used.

A recovery test was performed to determine the extraction efficiency of the method. For this purpose, different concentrations of ERG, TenA, AOH, and AME standards were added to TPU samples of known ERG, TenA, AOH, and AME concentrations. In Table 1, are given data on the linearity of the standard curve, limit of detection, recovery, and precision of the proposed method for the determination of ERG, TenA, and AME.

Table 1.

Statistical Analysis

Statistical analysis of the data was performed using IBM SPSS statistical analysis software for Windows version 23.0 (IBM Corp. 2015). The analysis of variance (ANOVA) revealed a significant effect ($p<0.05$), and the data means are compared with the least significant difference (LSD) test. Furthermore, bivariate correlations revealing a significant effect ($p<0.05$ and $p<0.01$) were examined.

RESULTS AND DISCUSSION

The relationship between ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations of TPO samples with different surface decay proportions

In the Table 2, are shown ERG (mg/kg) and *Alternaria* mycotoxin (TenA, AOH, and AME) (µg/kg) data for TPO samples, produced with decay proportion of: 25, 50, 75, and 100%. In the moldy tomato samples were detected *Alternaria* mycotoxins, including AOH, AME, and TenA, and these are in accordance with the statements of Hasan [46]. Nizamlıoğlu [26] reported that there is a linear relationship between ergosterol and *Alternaria* toxins depending on the decay rate, and that ergosterol is correlated with the AOH and TenA concentration in tomato paste. ERG and *Alternaria* mycotoxins concentrations in the TPU samples were significantly ($p<0.05$) affected by surface decay proportions (SDP).

Table 2.

There is a positive relationship between TSD proportions and ERG concentrations, as revealed by HPLC analysis in TPO samples. A similar trend is observed even for *Alternaria* mycotoxins concentrations measured. With the raising of TSD proportions, reaching till 100%, the ERG concentrations in TPO have been increased too, from an undetectable value to 8.40 mg/kg. Also, the same phenomena are observed for *Alternaria* mycotoxins (TenA, AOH, and AME), where from an undetected level till the TSD proportions reached 100%, such

concentrations reached respectively 4270, 5106, and 2500 µg/kg. A limited number of studies have been conducted in the literature on the AME, AOH and TA contents of tomato paste and tomato pulp. However, Ekinci et al., [29] similarly reported that Aflatoxin, patulin and ergosterol contents in hazelnuts had a linear correlation with different degradation rates. In a study, high levels of AOH and TA (>50 mg/kg) have been reported in tomatoes showing typical rot due to *Alternaria* decay [47].

The relationship between ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations in TPU samples, with different surface decay proportions

ERG and *Alternaria* mycotoxins concentrations in the TPU samples were significantly ($p<0.05$) affected by the SDP (Table 3). There is also a positive correlation between the ERG and *Alternaria* mycotoxins concentrations in TPU samples and the TSD proportions.

Table 3.

The concentration of ERG in TPU, ranged from 0.12 mg/kg to a value of 30.54 mg/kg from sound to 100% TSD, while for the *Alternaria* mycotoxins (TenA, AOH, and AME), have reached 21890, 25789, and 14680 µg/kg concentrations from 2, 3 and 0 µg/kg. Fliszár-Nyúl et al. [48] reported that AOH ranged from 6.1 to 25 µg/kg in tomato products. It was similar to the results obtained in TPU samples obtained from tomatoes with 0% TSD rate. In different studies conducted, the highest AOH and AME were reported in tomato purees, while the highest TenA was detected in tomato products [49]. Ergosterol has been used as a microbiological quality indicator in tomatoes and tomato products in recent years. It is reported that there is a limit value of 15 mg/kg for tomatoes and tomato products [28]. It is observed that TPU samples obtained from tomatoes with 75% and 100% TSD exceed the limit level of 15mg/kg ergosterol. EFSA reports that the average value for TA is a threshold of toxicological concern of 1500 ng/kg bw/day. AME and AOH in tomatoes have been reported to cause weak acute toxic effects to mice at 400 mg/kg (body weight) [50].

The relationship between some quality parameters of the TPU samples, with different surface decay proportions

The quality parameters (Table 4) observed in TPU samples were pH, ºBx, TA, and Hunter Lab color values, which were prepared from sound at 25, 50, 75, and 100% TSD.

Table 4.

As it is presented in Table 4, there is a slight increase ($p<0.05$) in pH and TA values of TPU samples produced using sound, 25, 50, 75, and 100% TSD.

The changes in the ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations in TPU and TPO samples, depending on different TSD proportions

The changes in the ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations in TPU and TPO samples, obtained from different TSD proportions, are expressed by trendlines and the coefficient of determination (R^2), which are displayed in Fig. 1 and Fig. 2, respectively.

Figure 1

Figure 2.

It is noted that the amounts of ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) in TPO samples are lower than in TPU samples. The data obtained at different TSD proportions reveal that these substances are transferred more during tomato processing in the TPU samples compared to the TPO samples. Furthermore, this transfer has shown an increasing trend, depending on the proportion of TSD.

The changes in the concentrations of *Alternaria* mycotoxins (TenA, AOH, and AME) depending on ERG concentrations in TPU and TPO samples

Figure 3.

The changes in the concentrations of *Alternaria* mycotoxins (TenA, AOH, and AME) depending on ERG concentrations in TPU and TPO samples are displayed in Figures 3 and 4. They are expressed by trendlines and coefficient of determination (R^2) values.

Figure 4.

The correlations and significance levels between the TSD proportions, the ERG concentration, and the *Alternaria* mycotoxins concentrations, and some quality parameters of TPU and TPO samples

In this study, the correlation method is used to examine the relationship status between variables and to determine how the other variable explains many variations within one variable. As shown in Figures 1, 2, 3, and 4, the coefficient of determination value is calculated from the correlation value. In Table 5, are presented the computed data for

correlations between the TSD proportions, the ERG concentration, and the *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations and some quality parameters (such as pH, TA, ºBx, and Hunter L*, a*, b* color values) for TPU and TPO samples and the significance levels. Results show that between all variables a positive relationship exists. The following results emerge according to the coefficient of determination of variables.

The correlation of the TSD proportions between ERG concentration in TPU, and TPO samples has no significant effects on the pH, TA, ºBx, Hunter L*, and a* color values. These findings are consistent with those of Kadakal et al. [40] study, except of Hunter b* color values, for which the significances are at the 0.01 level. The variations within TSD proportions in the TPU samples are explained by 93.3% for Hunter b* color values. The variations within ERG concentration in the TPU and TPO samples for Hunter b* color values are explained, respectively, by 96.6% and 99.0%.

The correlations of the TSD proportions are significant at the 0.01 level with ERG concentration, and AME is significant at the 0.05 level with TenA and AOH concentration in TPU samples. Variations within TSD proportions are explained by 97.6% for the concentration of ERG, and by 89.7% of TenA, 90.1% for AOH, and 92.4% of AME in TPU samples. The relationship between TSD proportions is significant with concentrations of ERG, TenA, and AOH at the 0.01 level, and AME at the 0.05 level in TPO samples. ERG concentration explains 96.4% of the changes in TSD rates. In TPO samples, 97.0% of TenA is accounted for by 97.2% of AOH and 91.6% of AME concentration. Graselli et al. [51] who stated essential correlations between ERG concentration and TSD.

It is noted that the concentrations of ERG and *Alternaria* mycotoxins in the TPU samples are related at the 0.05 significance level. The concentrations of 87.2% TenA, 87.2% AOH, and 91.0% AME in TPU samples are explained by the changes in ERG concentration in TPU. The concentrations of ERG and *Alternaria* mycotoxins in the TPO are related too. The relationship between the ERG concentration in the TPU samples is significant at the 0.01 level with TenA, AOH, and at the 0.05 level with the AME concentration in TPO. Variations in ERG concentration of TPU samples are explained by 99.2% of ERG, 98.0% of TenA, 98.2% of AOH, and 89.9% of AME in TPO samples. In the TPO samples, the concentrations of ERG and *Alternaria* mycotoxins are related at the 0.01 significance level. The variations within ERG concentration are explained, respectively, by 99.2% of TenA concentration, 99.4% of AOH concentration, and 92.9% of AME concentration in the TPO samples.

Nizamlıoğlu [26], investigated the relationship between ergosterol and three important mycotoxins (alternariol, alternariol monomethyl ether and tenuazonic acid) in tomato paste and tomato juice. The results revealed that AOH and TA toxins were associated with ergosterol in tomato paste and tomato juice.

Table 5.

CONCLUSION

In this study, the concentrations of ERG and *Alternaria* mycotoxins in TPO and TPU samples produced with decay proportion of: 25, 50, 75, and 100%, with different TSD proportions are measured. The relationships of these parameters with each other are estimated too.

In the moldy tomato samples were detected *Alternaria* mycotoxins, including AOH, AME, and TenA that is in accordance with similar studies. ERG and *Alternaria* mycotoxins concentrations in the TPU samples were significantly ($p<0.05$) affected by surface decayed proportions (SDP). There is a positive relationship between TSD proportions and ERG concentrations, as revealed by HPLC analysis in TPO samples. A similar trend is observed even for *Alternaria* mycotoxins concentrations measured. With the raising of TSD proportions, reaching till 100%, the ERG concentrations in TPO have been increased too. Also, the same phenomena are observed for *Alternaria* mycotoxins (TenA, AOH, and AME), where from an undetected level till the TSD proportions reached 100%. ERG and *Alternaria* mycotoxins concentrations in the TPU samples were significantly ($p<0.05$) affected by the SDP. There is also a positive correlation between the ERG and Alternaria mycotoxins concentrations in TPU samples and the TSD proportions. It is noted that the amounts of ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) in TPO samples are lower than in TPU samples. The reason why ERG, AME, AOH and TA contents in TPO samples are lower than in TPU samples is due to the high heat treatment applied during tomato paste production. The data obtained at different TSD proportions reveal that these substances are transferred more during tomato processing in the TPU samples compared to the TPO samples. Furthermore, this transfer has shown an increasing trend, depending on the proportion of TSD.

The correlations and the significance levels between the TSD proportions, the ERG, and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations and some quality parameters (pH, TA, ^{o}Bx, and Hunter L*, a*, and b* color values) shown that between all variables, a positive relationship exists for TPU and TPO samples. The correlation of the TSD proportions

between ERG concentration in TPU and TPO samples has no significant effects on the pH, TA, ºBx, Hunter L*, and a* color values. These findings are consistent with similiar studies.

Thus, this research study accomplishes the need that exist in defining the performance criteria for the analysis of *Alternaria* toxins in various foods and feeds, as stated in EFSA opinion. The high correlation percentages and correlation coefficients found in this work, strongly suggest that measurements of these parameters can be considered as quality indicators. Thus, it would be considered necessary for the evaluation of the defects and might be useful to be used by the tomato processing industry. The results of this study may serve as new data for future follow-up studies in this field.

CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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AUTHOR CONTRIBUTIONS

Çetin Kadakal: Methodology, Writing-original draft, Supervision. **Bilge Akdeniz:** Methodology, Formal analysis, Writing-review & editing, Visualization. **Ayten Ekinci:** Methodology, Supervision, Writing-review & editing. **Luziana Hoxha:** Conceptualization, Supervision, Writing-review & editing. **Pınar Şengün:** Supervision, Writing-review & editing

REFERENCES

1. S. Dagnas, J.M Membré, J. Food Prot. 76(3) (2013) 538-551. https://doi.org/10.4315/0362-028X.JFP-12-349

2. J.L. Richard, Int. J. Food Microbiol. 119(1-2) (2007) 3-10. https://doi.org/10.1016/j.ijfoodmicro.2007.07.019

3. R. Barkai-Golan, in Mycotoxins in fruits and vegetables, R. Barkai-Golan, N. Paster Ed., Elsevier Inc, Amsterdam (2008) 185-204. https://doi.org/10.1016/B978-0-12-374126-4.X0001-0

4. R.A. Marc, in Mycotoxins and Food Safety-Recent Advances, R.A. Marci Ed., IntechOpen, Romania (2022) p. 149. https://doi.org/10.5772/intechopen.95720

5. J.I Pitt, M.H. Taniwaki, M.B. Cole, Food Control, 32(1) (2013) 205-215. https://doi.org/10.1016/j.foodcont.2012.11.023

6. S.D. Motta, L.M. Valente-Soares, Food Addit. Contam. 18(7) (2001) 630-634. https://doi.org/10.1080/02652030117707

7. V.E.F. Pinto, A. Patriarca, in Mycotoxigenic Fungi: Methods and Protocols, M. Antonio, S. Antonio Ed., Humana Press, New Jersey (2017) p. 394. https://doi.org/10.1007/978-1-4939-6707-0_1

8. EFSA, EFSA on Contaminants in the Food Chain, EFSA J. 9 (10) (2011) 2407. https://doi.org/10.2903/j.efsa.2011.2407.

9. L.S. Jackson, F. Al-Taher, in Mycotoxins in fruits and vegetables, R. Barkai-Golan, N. Paster Ed., Elsevier Inc. Amsterdam (2008) 75-104. https://doi.org/10.1016/B978-0-12-374126-4.X0001-0.

10. L. Escrivá, S. Oueslati, G. Font, L. Manyes, J. Food Qual. 32(1) (2017) 205-215. https://doi.org/10.1016/j.foodcont.2012.11.023

11. M. Solfrizzo, Curr. Opin. Food Sci. 17 (2017) 57-61. https://doi.org/10.1016/j.cofs.2017.09.012

12. Y. Ackermann, V. Curtui, R. Dietrich, M. Gross, H. Latif, E. Märtlbauer, E. Usleber, J. Agric. Food Chem. 59(12) (2011) 6360-6368. https://doi.org/10.1021/jf201516f

13. J. Noser, P. Schneider, M. Rother, H. Schmutz, Mycotoxin Res. 27(4) (2011) 265-271. https://doi.org/10.1007/s12550-011-0103-x

14. N. Jiand, Z. Li, L. Wang, H. Li, X. Zhu, X. Feng, M. Wang, Int. J. Food. Microbiol. 311 (2019) 108333. https://doi.org/10.1016/j.ijfoodmicro.2019.108333

15. V. Ostry, World Mycotoxin J. 1(2) (2008) 175-188. https://doi.org/10.3920/WMJ2008.x013
16. C. Juan, L. Covarelli, G. Beccari, V. Colasante, J. Mañes, Food Control, 62 (2016) 322-329. https://doi.org/10.1016/j.foodcont.2015.10.032
17. K. Sivagnanam, E. Komatsu, C. Rampitsch, H. Perreault, T. Gräfenhan, J. Sci. Food Agric. 97(1) (2017) 357-361. https://doi.org/10.1002/jsfa.7703
18. M. Zachariasova, Z. Dzuman, Z. Veprikova, K. Hojkova, M. Jiru, M. Vaclavikova, A. Zachariasova, M. Pospichalova, M. Florian, J. Hajslova, Anim. Feed Sci. Technol. 193 (2014) 124-140. https://doi.org/10.1016/j.anifeedsci.2014.02.007
19. W. Jia, X. Chu, Y. Ling, J. Huang, J. Chang, J. Chromatogr. A. 1345 (2014) 107-114. https://doi.org/10.1016/j.chroma.2014.04.021
20. W. A. Abia, B. Warth, M. Sulyok, R. Kriska, A.N. Tchana, P.B. Njobeh, M.F. Dutton, P.F. Moundipa, Food Control, 31(2) (2013) 438-453. https://doi.org/10.1016/j.foodcont.2012.10.006
21. P. López, D. Venema, T. Rijk, A. Kok, J.M. Scholten, H.G.J. Mol, M. Nijs, Food Control, 60 (2016) 196-204. https://doi.org/10.1016/j.foodcont.2015.07.032
22. S. Hickert, M. Bergmann, S. Ersen, B. Cramer, H.U. Humpf, Mycotoxin Res. 32(1) (2016) 7-18. https://doi.org/10.1007/s12550-015-0233-7
23. V.M. Scussel, J.M. Scholten, P.M. Rensen, M.C. Spanjer, B.N.E. Giordano, G.D. Savi, Int. J. Food Sci. Technol. 48(1) (2013) 96-102. https://doi.org/10.1111/j.1365-2621.2012.03163.x
24. A. Logrieco, A. Moretti, M. Solfrizzo, World Mycotoxin J. 2(2) (2009) 129-140. https://doi.org/10.3920/WMJ2009.1145
25. M. Meena, A. Zehra, P. Swapnil, M.K. Dubey, C.B. Patel, R.S. Upadhyay, Arch. Phytopathol. Plant Prot. 50(7-8) (2017) 317-329. https://doi.org/10.1080/03235408.2017.1312769
26. N.M. Nizamlıoğlu, J. Food Process. Preserv. 46 (11) (2022) e16937. https://doi.org/10.1111/jfpp.16937
27. M. Eskola, A. Altieri, J.J.W.M.J. Galobart, World Mycotoxin J. 11(2) (2018) 277-289. https://doi.org/10.3920/WMJ2017.2270
28. Ç. Kadakal, T.K. Tepe, Food Rev. Int. 35 (2) (2019) 155-165. https://doi.org/10.1080/87559129.2018.1482495
29. R. Ekinci, Ç. Kadakal, M. Otağ, J. Food Prot. 77 (3) (2014) 499-503. https://doi.org/10.4315/0362-028X.JFP-13-215

30. Ç. Kadakal, PhD Thesis, Ankara University, Institute of Science, Ankara, (2003) Turkey.
31. Ç. Kadakal, N. Artık, Crit. Rev. Food Sci. Nutr. 44 (5) (2004) 349-351. https://doi.org/10.1080/10408690490489233
32. Ç. Kadakal, S. Nas, R. Ekinci, Food Chem. 90 (2005) 95-100. https://doi.org/10.1016/j.foodchem.2004.03.030
33. S. Marin, D. Cuevas, A.J. Ramos, V. Sanchis, Int. J. Food Microbiol. 121 (2008) 139-149. https://doi.org/10.1016/j.ijfoodmicro.2007.08.030
34. M.H. Taniwaki, A.D. Hocking, J.I. Pitt, G.H. Fleet, Int. J. Food Microbiol. 68 (2001) 125-133. https://doi.org/10.1016/S0168-1605(01)00487-1
35. S. Bermingham, L. Maltby, R.C. Cooke, Mycol. Res. 99 (1995) 479-484. https://doi.org/10.1016/S0953-7562(09)80650-3
36. H. Gourama, L.B. Bullerman, J. Food Prot. 558 12 (1995) 1395–1404. https://doi.org/10.4315/0362-028X-58.12.1395.
37. Ç. Kadakal, S. Nas, R. Ekinci, Food Chem. 90 (2005) 95-100. https://doi.org/10.1016/j.foodchem.2004.03.030
38. Ç. Kadakal, M.N. Nizamlıoğlu, T.K. Tepe, S. Arısoy, B. Tepe. H.S. Batu, Turk. J. Agric. Food Sci. Tech. 8 (4) (2020). https://doi.org/10.24925/turjaf.v8i4.895-900.3071
39. M.R. Zill, J.E. Ehgelhardt, P.R. Wallnofer, Zeitschrift fur Lebensmittel-untersuchung und -forschung, 15 (1988) 20-22. https://doi.org/10.1007/bf01043094
40. Ç. Kadakal, Ş. Tağı, N. Artık, J. Food Qual. 27 (4) (2004) 255-263. https://doi.org/10.1111/j.1745-4557.2004.00631.x
41. AOAC, Methods, Assoc. Off. Anal. Chem, 15th Ed., Arlington (1990) p. 910. ISBN 0-935584-40. https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf
42. L.J. Mauer, R.L. Bradley, in Food Analysis, S.S. Nielsen Ed., Springer, New York (2017) 257-286. https://doi.org/10.1007/978-3-319-45776-5_15
43. C. Tyl, G.D. Sadler, in Food Analysis, S.S. Nielsen Ed., Springer, New York (2017) 389-406. https://doi.org/10.1007/978-3-319-45776-5_22
44. R.E. Wrolstad, D.E. Smith, in Food Analysis, S.S. Nielsen Ed., Springer, New York (2017) 545-555. https://doi.org/10.1007/978-3-319-45776-5_31
45. L. Terminiello, A. Patriarca, G. Pose, V.F. Pinto, Mycol. Res. 22 (4) (2006) 236-240. https://doi.org/10.1007/BF02946748
46. H.A. Hasan, Acta Microbiol. Immunol. Hung. 43 (2-3) (1996) 125-133. https://doi.org/10.1007/BF01103101

47. J. Walravens, H. Mikula, M. Rychlik, S. Asam, T. Devos, E.E. Njumbe, D.D. Mavungu, J. Jacxsens, L. Van, A. Landschoot, L. Vanhaecke, S. Saeger, J. Agric. Food Chem. 64(24) (2016) 5101-5109. https://doi.org/10.1021/acs.jafc.6b01029
48. E. Fliszár-Nyúl, Á. Szabó, L. Szente, M. Poór, J. Mol. Liq. 319 (2020) 114180. https://doi.org/10.1016/j.molliq.2020.114180
49. J.D. Ioi, PhD Thesis, The University of Guelph, Department of Food Science, Ontario, (2017) Canada.
50. S.D. Motta, L.M.V. Soares, Braz. J. Microbiol. 31(4) (2000) 315-320. https://doi.org/10.1590/S1517-83822000000400015
51. C. Graselli, C. Leoni, C. Sandei, G. Mori, Ind. Conserve, 68 (1993) 1-10. http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=4859569

Table Captions

Table 1. Characteristics of the proposed method: linearity of calibration curve, limit of detection, recovery, and precision

Table 2. The relationships of ERG and *Alternaria* mycotoxins concentrations in TPO samples, with different surface decay proportions

Table 3. Relationship between ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations in TPU samples, with different surface decay proportions

Table 4. Some quality parameters of the TPU samples, prepared from sound and 25, 50, 75, and 100% TSD

Table 5. Correlations and significance levels between the TSD proportions, the concentrations of ERG and *Alternaria* mycotoxins, and some quality parameters of TPU and TPO samples

Figure Legends

Fig. 1. Changes in the concentrations of ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) of TPU samples with different TSD proportions, expressed by the trendlines and the coefficient of determination (R^2).

Fig. 2. Changes in the concentrations of ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) in TPO samples, with different TSD proportions, expressed by trendlines and the coefficient of determination (R^2)

Fig. 3. The changes in the concentrations of *Alternaria* mycotoxins (TenA, AOH, and AME) with different ERG concentrations in TPU samples, expressed by trendlines and the coefficient of determination (R^2)

Fig. 4. Changes in the concentrations of *Alternaria* mycotoxins (TenA, AOH, and AME) with different ERG concentrations of TPO samples are given by trend lines, and the coefficient of determination (R^2)

Table 1. Characteristics of the proposed method: linearity of calibration curve, limit of detection, recovery, and precision

Alternaria Toxins and ERG	Linear range (µg/L)	R	R^2	Detection limit (µg /kg)	Recovery (%) Mean SD[b]	Precision R.S.D. (%)
TenA	5.0-30000	0.9994	99.71	2.2	92.86 ± 0.9	5.7
AOH	1.0-30000	0.9990	99.90	0.8	95.40 ± 1.0	4.1
AME	1.0-30000	0.9976	99.88	1.1	92.60 ± 1.1	2.34
ERG	1-25000	0.9999	99.93	1.0	94.50 ± 1.0	3.68

Mean SD[b]: Mean ± standard deviation.

R.S.D: Relative standard deviation.

Table 2. The relationships of ERG and *Alternaria* mycotoxins concentrations in TPO samples, with different surface decay proportions

		Alternaria Mycotoxins		
TSD proportion (%)	ERG (mg/kg)[x]	TenA (µg/kg)[x]	AOH (µg/kg)[x]	AME(µg/kg)[x]
0	nd[a]*	nd[a]*	nd[a]*	nd[a]*
25	0.4[b]	340[b]	426[b]	120[b]
50	3.54[c]	2190[c]	2582[c]	1364[c]
75	5.68[d]	2980[cd]	3620[d]	2468[d]
100	8.40[e]	4270[d]	5106[e]	2500[d]

nd: not detected

* Values within the column with different letters are statistically significant ($p<0.05$).

[x] Mean values of ten determinations with two replicates.

Table 3. Relationship between ERG and *Alternaria* mycotoxins (TenA, AOH, and AME) concentrations in TPU samples, with different surface decay proportions

TSD proportions (%)	ERG (mg/kg)[x]	*Alternaria* Mycotoxins		
		TenA (µg/kg)[x]	AOH (µg/kg)[x]	AME(µg/kg)[x]
0	0.12[a]*	2[a]*	3[a]*	0[a]*
25	3.82[b]	1545[b]	1780[b]	682[b]
50	12.23[c]	16314[c]	18912[c]	9672[c]
75	19.80[d]	18356[d]	22768[d]	12230[d]
100	30.54[e]	21890[e]	25789[e]	14680[e]

*Values within a column followed by the different letters are significant ($p<0.05$).
[x] Values are the mean of ten determinations in two replicates.

Table 4. Some quality parameters of the TPU samples, prepared from sound and 25, 50, 75, and 100% TSD

TSD proportions (%)	pH[x]	TA (g/L)[x]	ºBx[x]	Hunter		
				L[x]	a[x]	b[x]
0	4.20[a]*	0.368[a]*	4.88[a]*	27.42[a]*	13.56[a]*	8.22[a]*
25	4.37[b]	0.352[b]	5,15[b]	27.78[a]	13.50[a]	8.18[a]
50	4.35[b]	0.364[a]	5.32[c]	29.42[b]	13.48[a]	8.60[b]
75	4.35[b]	0.375[c]	5,16[b]	30.26[c]	13.60[a]	8.90[cd]
100	4.30[c]	0.384[d]	4.90[a]	31.56[d]	13.98[b]	9.141[d]

* Values within the column with different letters are statistically significant ($p<0.05$).
[x] Average of ten determinations with two replicates.

Table 5
Correlations and significance levels between the TSD proportions, the concentrations of ERG and *Alternaria* mycotoxins, and some quality parameters of TPU and TPO samples

		TPU										TPO			
		pH	TA	ºBx	Hunter L	Hunter a	Hunter b	ERG	TenA	AOH	AME	ERG	TenA	AOH	AME
TSD (%)	R	0.414	0.725	0.042	-0.352	0.726	0.966**	0.988**	0.947*	0.949*	0.961**	0.982**	0.985**	0.986**	0.957*
	p	0.489	0.166	0.946	0.561	0.165	0.007	0.002	0.015	0.014	0.009	0.003	0.002	0.002	0.010
	R²	**0.171**	**0.526**	**0.002**	**0.124**	**0.527**	**0.933**	**0.976**	**0.897**	**0.901**	**0.924**	**0.964**	**0.970**	**0.972**	**0.916**
TPU ERG		0.274	0.810	-	-0.430	0.810	0.983**		0.934*	0.934*	0.954*	0.996**	0.990**	0.991**	0.948*
	R			0.094											
	p	0.655	0.097	0.881	0.470	0.096	0.003		0.020	0.020	0.012	0.000	0.001	0.001	0.014
	R²	**0.075**	**0.656**	**0.009**	**0.185**	**0.656**	**0.966**		**0.872**	**0.872**	**0.910**	**0.992**	**0.980**	**0.982**	**0.899**
TPO ERG		0.242	0.837	-	-0.503	0.788	0.995**						0.996**	0.997**	0.964**
	R			0.081											
	p	0.695	0.077	0.897	0.388	0.113	0.000						0.000	0.000	0.008
	R²	**0.059**	**0.701**	**0.007**	**0.253**	**0.622**	**0.990**						**0.992**	**0.994**	**0.929**

R: correlation; R²: coefficient of determination; p: significance ; ºBx: Brix; TA: Titratable acidity.
**: Correlation is significant at the 0.01 level (2-tailed).
*: Correlation is significant at the 0.05 level (2-tailed).

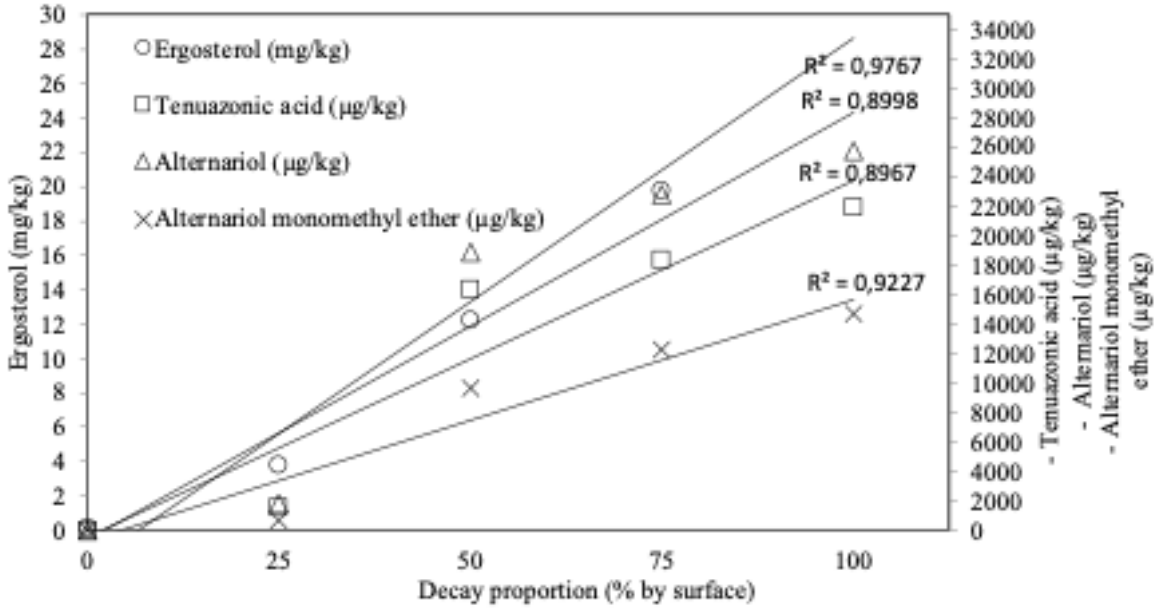
Ergosterol (mg/kg)
Tenuazonic acid (µg/kg)
Alternariol (µg/kg)
Alternariol monomethyl ether (µg/kg)
R² = 0,9767
R² = 0,8998
R² = 0,8967
R² = 0,9227
Ergosterol (mg/kg)
- Tenuazonic acid (µg/kg)
- Alternariol (µg/kg)
- Alternariol monomethyl ether (µg/kg)
Decay proportion (% by surface)
0
25
50
75
100

Fig. 1

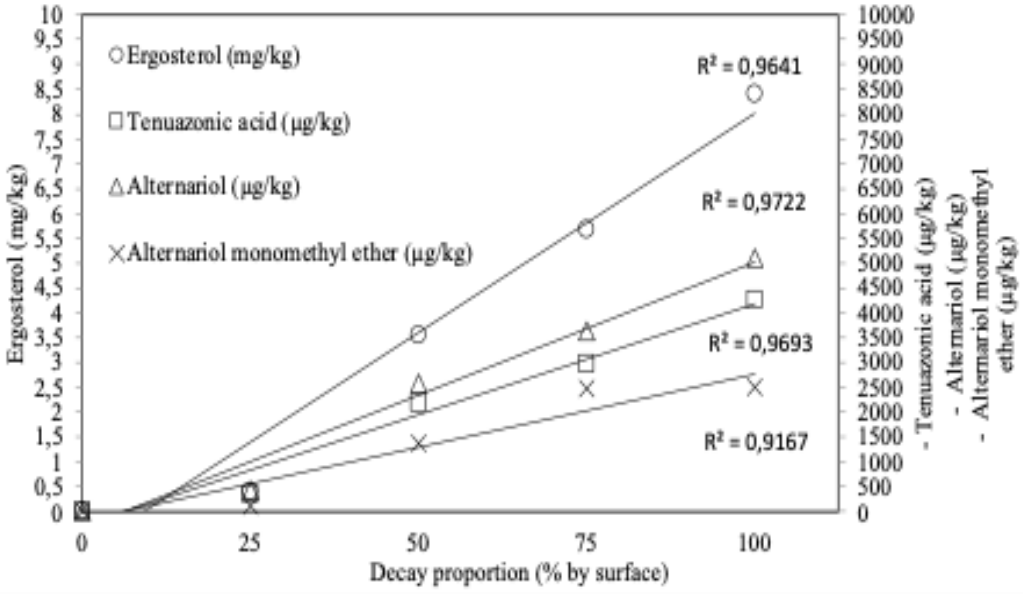
Ergosterol (mg/kg)
Tenuazonic acid (µg/kg)
Alternariol (µg/kg)
Alternariol monomethyl ether (µg/kg)
R² = 0,9641
R² = 0,9722
R² = 0,9693
R² = 0,9167
Ergosterol (mg/kg)
- Tenuazonic acid (µg/kg)
- Alternariol (µg/kg)
- Alternariol monomethyl ether (µg/kg)
Decay proportion (% by surface)

Fig. 2

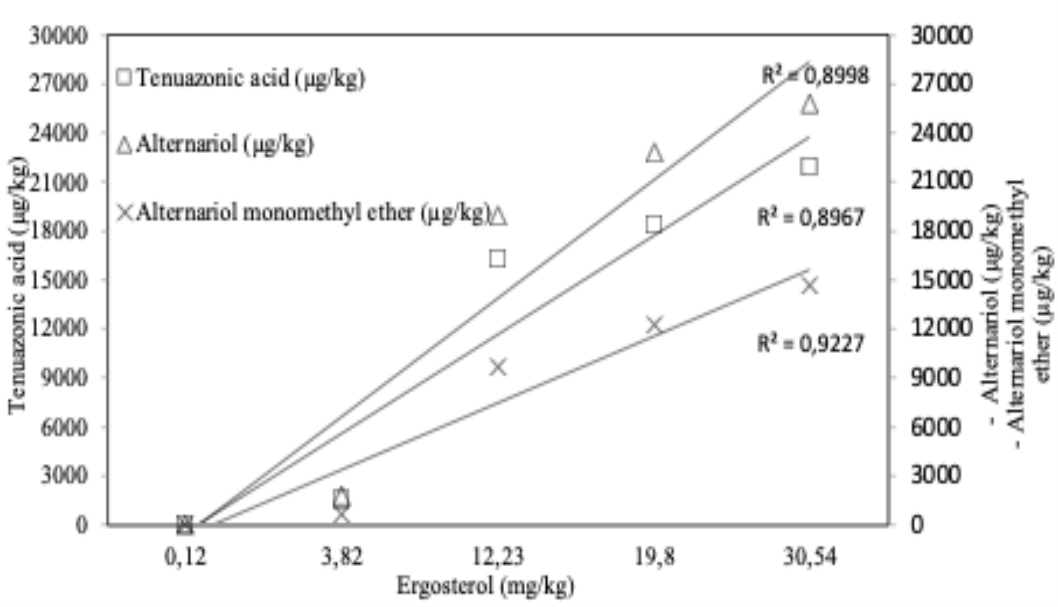
Tenuazonic acid (µg/kg)
Alternariol (µg/kg)
Alternariol monomethyl ether (µg/kg)
R² = 0,8998
R² = 0,8967
R² = 0,9227
Tenuazonic acid (µg/kg)
- Alternariol (µg/kg)
- Alternariol monomethyl ether (µg/kg)
Ergosterol (mg/kg)
0,12
3,82
12,23
19,8
30,54
0
3000
6000
9000
12000
15000
18000
21000
24000
27000
30000

Fig. 3

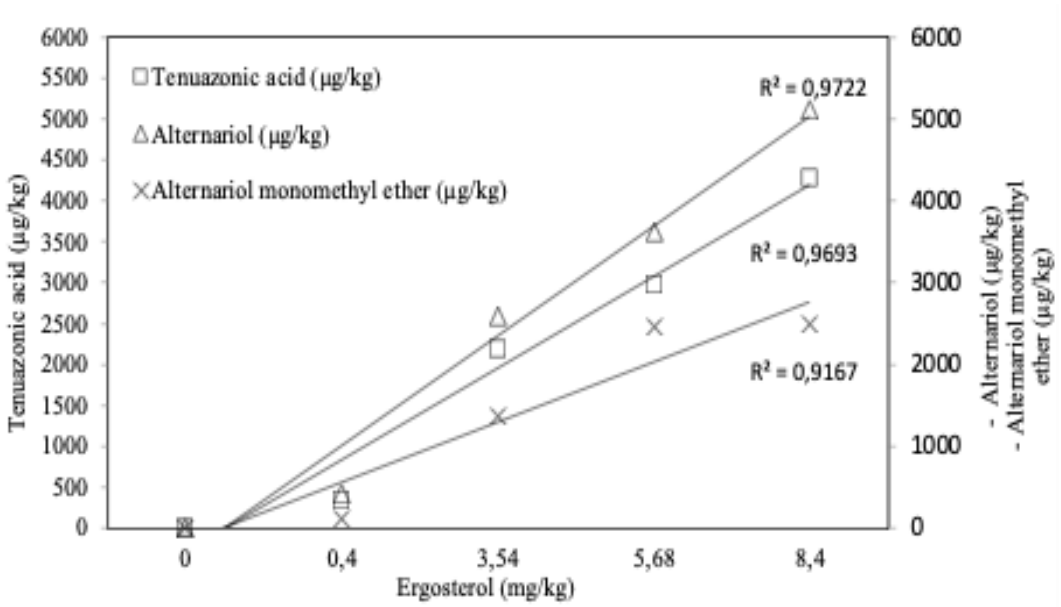
Tenuazonic acid (µg/kg)
Alternariol (µg/kg)
Alternariol monomethyl ether (µg/kg)
R² = 0,9722
R² = 0,9693
R² = 0,9167
Tenuazonic acid (µg/kg)
- Alternariol (µg/kg)
- Alternariol monomethyl ether (µg/kg)
Ergosterol (mg/kg)
0
0,4
3,54
5,68
8,4
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0

Fig. 4.