Food Control 145 (2023) 109436

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Rosmarinus officinalis reduces the ochratoxin A production by *Aspergillus westerdijkiae* in a dry-cured fermented sausage-based medium☆

Micaela Álvarez [a], María J. Andrade [a,*], Josué Delgado [a,**], Félix Núñez [a], Ángel-Carlos Román [b], Paula Rodrigues [c]

[a] Grupo de Investigación Higiene y Seguridad Alimentaria, Instituto de Investigación de Carne y Productos Cárnicos (IProCar), Facultad de Veterinaria, Universidad de Extremadura, Avda. de las Ciencias, s/n, 10003, Cáceres, Spain
[b] Departamento de Bioquímica, Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Avda. de Elvas, s/n, 06071, Badajoz, Spain
[c] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal

ARTICLE INFO

Keywords:
Biocontrol
Rosemary
Ochratoxigenic fungi
Meat products
Proteomics

ABSTRACT

Aspergillus westerdijkiae is an ochratoxin A (OTA) producer mould in dry-cured meat products. To prevent the presence of ochratoxigenic moulds in dry-cured fermented sausages, natural strategies based on the use of herbs and their derivatives are in the spotlight. The aim of this study was to test the potential antiochratoxigenic effects of rosemary leaves (R) and rosemary essential oil (REO) as biocontrol agents (BCAs) in a dry-cured fermented sausage-based medium. The mechanisms involved in their effects were also analysed by Proteomics. A control without BCAs and three treatments (R, REO and the combination of R + REO) were carried out. No significant differences were detected when BCAs were individually applied, whilst a significant reduction up to 73.87% in OTA was provoked by R + REO. This combination showed a synergistic effect, in which proteins from PKS ER domain and cell wall integrity pathway seem to be involved. REO alone exerted similar effect on the mould proteome than R + REO, but in a lesser extent. The impact of R in this synergy has not been completely elucidated. The combination of R + REO together with other strategies could minimise the hazard posed by *A. westerdijkiae* in dry-cured fermented sausages.

1. Introduction

The most found mycotoxin in dry-cured fermented sausages is ochratoxin A (OTA), named after the first identified mould in which it was detected, *Aspergillus ochraceus*. However, this isolate was ulteriorly renamed as *Aspergillus westerdijkiae* (Frisvad et al., 2004), suggesting that many *A. ochraceus* have been incorrectly identified. Although *Penicillium nordicum* has been traditionally considered as the main responsible for the presence of OTA in dry-cured meat products (Battilani et al., 2007; Ferrara et al., 2016), *A. westerdijkiae* is able to produce much higher mycotoxin concentrations than *P. nordicum* under the same conditions, being also recognised as an important OTA producer in dry-cured fermented meat products (Merla et al., 2018; Parussolo et al., 2019; Rodrigues et al., 2019; Vipotnik et al., 2017). Such mycotoxin is nephrotoxic, immunotoxic, neurotoxic and teratogenic (EFSA CONTAM Panel et al., 2020). Furthermore, it has been classified in Group 2B as possibly carcinogenic to humans (IARC, 1993), and even recent studies have considered its reclassification in the Group 2A as probably carcinogenic (Ostry et al., 2017).

To prevent OTA contamination in dry-cured fermented sausages the use of antifungal strategies is essential. Natural and environmentally friendly antifungals are gaining importance to reduce the synthetic compounds in the food industry. Concretely, several herbs and spices have shown antimicrobial properties to extend foodstuff shelf life (Sánchez-Montero et al., 2019; Tajkarimi et al., 2010). Some of them, like rosemary, oregano, paprika and black pepper, are frequently added as ingredients to dry-cured fermented sausages, contributing to their characteristic flavour (García-Fontán et al., 2007; Lozano-Ojalvo et al., 2015). Herbs, spices and their derivatives (essential oils (EOs) and phenolic extracts) have been tested against foodborne ochratoxigenic

☆ The aim of this study was to evaluate the potential use of R, REO and their combination as BCAs against *A. westerdijkiae* by checking their effect on the OTA production as well as on the *A. westerdijkiae* proteome in a dry-cured fermented sausage-based medium.

* Corresponding author.
** Corresponding author.
E-mail addresses: mjandrad@unex.es (M.J. Andrade), jdperon@unex.es (J. Delgado).

https://doi.org/10.1016/j.foodcont.2022.109436
Received 24 May 2022; Received in revised form 22 September 2022; Accepted 4 October 2022
Available online 8 October 2022

moulds (Álvarez et al., 2021a, 2020; Dammak et al., 2019; El Khoury et al., 2016, 2017). EO from basil totally inhibited the OTA production in *A. westerdijkiae* in Czapek Yeast Autolysate Agar (CYA; Císarová et al., 2020). Oregano EO successfully decreased OTA production by *Penicillium verrucosum* in yeast extract broth (Schlösser & Prange, 2019) but stimulated that found for *A. westerdijkiae*. EOs from rosemary (REO) and thyme reduced OTA generated by *Aspergillus carbonarius* in a synthetic grape medium (El Khoury et al., 2016). The REO has also demonstrated to diminish the OTA generation by *A. ochraceus* and *A. westerdijkiae* in CYA (Císarová et al., 2020) and rosemary leaves (R) dropped that from *P. nordicum* in dry-cured fermented sausages (Álvarez et al., 2022a). Synergism between some extracts has been described in different studies (Tajkarimi et al., 2010). The antifungal and antiochratoxigenic activities of French lavender and laurel EOs against *A. carbonarius* have been attributed to the synergistic effect of some of their minor components with the major component 1,8-cineole (Dammak et al., 2019). Thus, the study of synergistic combinations of biocontrol agents (BCAs), including their main modes of action, would entail to maximise their antifungal effects.

To elucidate the modes of action of BCAs and their effects on the physiology of toxigenic moulds, proteomic analyses can be performed. This technique has been applied to study the targets of microbial antifungal BCAs, such as *Debaryomyces hansenii*, *Penicillium chrysogenum* and *Candida intermedia* (Álvarez et al., 2022a, 2022b; Delgado et al., 2019; Tilocca et al., 2019), as well as herbs and their derivatives (Hu et al., 2022; Lai et al., 2021; Álvarez, Delgado, et al., 2022). This methodology has allowed to attribute the growth inhibition of *Aspergillus flavus* by perilla EO to the blockage of the antioxidative response and the glycolysis pathway (Hu et al., 2022). Cinnamon EO limited the *Penicillium expansum* growth by disturbing the carbohydrate metabolic process (Lai et al., 2021). R decreased the OTA and its precursors, and the abundance of proteins involved in ergosterol biosynthesis in *P. nordicum* in dry-cured fermented sausages (Álvarez, Delgado, et al., 2022). In addition, REO *in vitro* dropped the OTA concentration and lowered the amount of proteins related to the polyketide synthase enoylreductase domain (PKS ER) in *P. nordicum*, involved in the OTA biosynthesis (Álvarez et al., 2021a).

2. Materials and methods

2.1. Microorganisms and plant material

The *A. westerdijkiae* OTA-producing strain was isolated from dry-cured ham (Rodrigues et al., 2019) and is deposited in the fungal culture collection Micoteca da Universidade do Minho (Braga, Portugal) under the code MUM 16.142.

Rosemary was collected in the region of Extremadura (Spain). REO was extracted by Clevenger hydrodistillation as described by Álvarez et al. (2020). The oily phase was separated and kept at −20 °C. REO was then dissolved in water with 1% (v/v) of Tween 80 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) up to a final concentration of REO:water 1:1.

2.2. Culture media

A. westerdijkiae was grown on Potato Dextrose Agar (PDA; Biolife, Milan, Italy).

Dry-cured fermented sausage-based medium was prepared as described by Álvarez et al. (2020) and supplemented with 2 g of R per kg of lyophilised "salchichón" in the corresponding treatments, an amount commonly added during the manufacturing of dry-cured fermented sausages (0.2% w/w; Vignolo et al., 2010).

All the media were autoclaved for 15 min at 121 °C, cooled at 50 °C and poured into Petri plates.

2.3. Inoculation and experimental settings

Suspension of *A. westerdijkiae* conidia was prepared from cultures in PDA incubated for 7 days at 25 °C. The spores were counted in a Neubauer chamber (Labor Optik, Lancing, UK) using a microscope model LaborLux 12 (Leitz, Stuttgart, Germany) and adjusted to a final concentration of 10^6 spores/mL of phosphate buffer saline (VWR Chemicals, Radnor, Pennsylvania, USA).

A collagen casing with an area similar to the Petri plates surface (≈63.6 cm^2), previously sterilised by immersion in ethanol and exposed to UV light for 1 day, was placed onto the medium surface to simulate the physical conditions in the real sausage. Four different batches were prepared: control without BCAs (C); treatment R with the addition of R in the medium; treatment REO with 100 μL of REO applied on the surface of the casings (effective concentration against *P. nordicum* described by Álvarez et al. (2021a) and treatment R + REO combining both BCAs in the amounts specified in the above described individual treatments. Finally, 2 μL of *A. westerdijkiae* 10^6 spores/mL inoculum were added to the centre of each casing. The plates were incubated for 3 days at 22 °C and 90% relative humidity (RH) and the following 12 days at 15 °C and 85% RH to simulate the industrial ripening of dry-cured fermented sausages. The treatments were performed in triplicate.

2.4. OTA extraction and analysis

After the incubation period, a half of the mycelium was collected with a sterile scalpel and transferred into 45 mL plastic tube. The samples were stored at −20 °C until performing the OTA extraction using QuEChERs methodology (Delgado et al., 2019). The samples were filtered through a 0.22 μm polytetra-fluorethylene (PTFE) membrane (FiltraTech, Saint Jean de Braye, France) before analysis.

OTA was analysed using a High-Performance Liquid Chromatography (HPLC) Smartline pump 1000 (Knauer, Berlin, Germany) coupled with a fluorescence detector FP-2020 (Jasco, USA). A C18 reverse-phase column PLRP-S 300 Å (250 × 4.6 mm, 8 μm, Polymer Laboratories, Church Stretton, UK) at 35 °C was used. The mobile phase composed by water:acetonitrile:acetic acid (29.5:70:0.5) was pumped in an isocratic mode at 0.8 mL/min. The injection volume was 20 μL. The OTA was detected at 330 nm and 463 nm excitation and emission wavelengths, respectively. The total run time was 15 min. The limit of detection (LOD) and the limit of quantification (LOQ) were 3 and 9 ng/mL, respectively, after calculating as described in Vipotnik et al. (2017).

2.5. Synergistic effect evaluation

The synergy between treatments was analysed using the Abbot formula described by Moreno et al. (2003):

$$Ie = X + Y - (XY/100)$$

Ie is the expected percentage of inhibition of a combination treatment, *X* and *Y* are the percentage of inhibition for each treatment separately and *XY* the percentage of inhibition for the combination treatment. The nature of this interaction was determined by the ratio (IR) where IR= Io/Ie. *Io* is the observed percentage of inhibition. A ratio up to 1.5 shows a synergistic interaction.

2.6. Comparative proteomic analyses

The proteomic analysis was carried out following the method described by Álvarez et al. (2021a). Briefly, around 200 mg of mycelium from each treatment were flash-frozen in liquid nitrogen and stored at −80 °C until extraction. The mycelium samples were lysed and homogenised using the FastPrep-24™ (Thermo Fisher Scientific) before sonication in a Branson sonifier™ 250 (Emerson, Barcelona, Spain). Lysates were then partially run in an SDS-PAGE and undergone to a

reduction-alkylation with dithiothreitol and iodoacetoamide (Promega, Madison, Wisconsin, USA). After digesting with trypsin (Promega) and ProteaseMAX (Promega), around 1 μg from every sample was analysed using a Q-Exactive Plus (Thermo Fisher Scientific) coupled to a Dionex Ultimate 3000 RSLCnano (Thermo Fisher Scientific).

Top15 method for MS/MS scans was used to collect the data (Delgado et al., 2019). Label-free comparative proteome abundance and data analysis were performed using MaxQuant software (v. 1.6.15.0; Cox & Mann, 2008) and Perseus software (v. 1.6.14.0) to organise the data. An annotated database of *A. westerdijkiae* proteins was built for comparison against the obtained peptides. The *A. westerdijkiae* genome database was obtained from Han et al. (2016). Their homolog proteins were specifically identified in *A. flavus*, *A. carbonarius* and *P. nordicum* using Blast (*e*-value<0.0001) and the annotations (protein name and description) were combined in a single FASTA file with *ad hoc* PERL scripts.

The maximum peptide/protein false discovery rates (FDR) were set to 1% and every protein was identified by at least two peptides. Only proteins detected in at least two replicates from at least one treatment were used in the analysis. The *t*-Test was applied to quantitative analysis between treatments ($p < 0.05$). Qualitative analysis was carried out by detecting proteins in at least two replicates of the same batch but in none of the compared batch. The enrichment analyses were carried out using ClueGO software (v. 2.5.6; Bindea et al., 2009). The Kappa score was set at 0.4 value and the *p*-value was analysed by Bonferroni step down and established at $p \leq 0.05$.

2.7. Statistical analysis

The statistical analysis for OTA production was carried out using IBM SPSS v. 20 (Armonk, New York, USA). Non-parametric tests (Kruskal-Wallis and Mann-Whitney U) were performed due to the non-normal distribution of the data (Shapiro-Wilk test). The statistical significance was established at $p \leq 0.05$.

3. Results and discussion

3.1. Mycotoxin production

The OTA concentration found in the control without BCAs (C) was surprisingly high, reaching values of 180,217.71 ± 82,004.03 ng/g of agar (shown as value 0% in Fig. 1). Previous studies have described the high capacity of *A. westerdijkiae* for producing OTA in a variety of conditions in dry-cured ham-based medium and salami, being the highest amounts (1934 and 691 ng/g, respectively) much lower than those found in the present study (Merla et al., 2018; Vipotnik et al., 2017). These differences can be due to the distinct composition of the meat substrates, the physicochemical values reached by the environmental factors and the variable capability of the studied strains to produce OTA. These results indicate the good adaptation of the tested strain isolated from dry-cured ham to a dry-cured fermented sausage-based substrate and its potential hazard when colonising meat products. The concentration found in our study exceeded more than 100,000 times the limit used as a reference, established in the Italian legislation (1 ng/g; Ministero Ministerio della Sanità, 1999), posing a high risk for the human health.

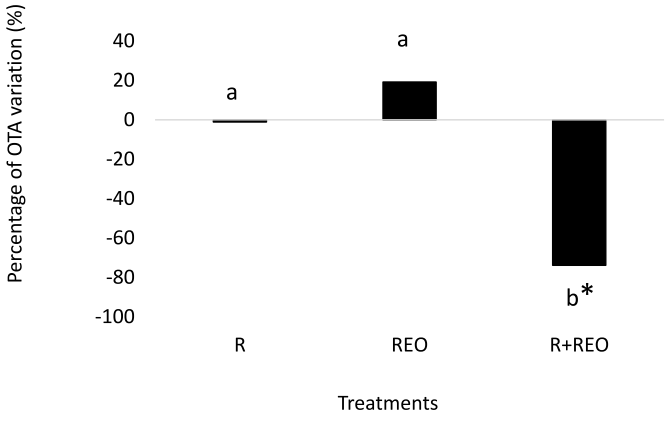

Fig. 1. Percentage of ochratoxin A (OTA) variation when applying biocontrol agents (BCAs) in a dry-cured fermented sausage-based medium (FS) against *Aspergillus westerdijkiae* MUM 16.142. It is expressed as percentage of OTA compared to the control (mould grown in the absence of BCAs, value = 0%) Treatments: R: Rosemary leaves (0.2%, w/w); REO: Rosemary essential oil applied on the surface of collagen casings over FS (100 μL). R + REO: combination of R and REO. Statistically different groups ($p \leq 0.05$) were indicated with different letters, and differences with respect to the control treatment with an asterisk. The experiment was performed in triplicate.

The R and the REO treatments individually applied had no significant reduction on the OTA production (Fig. 1). However, when both BCAs were combined, a significant decrease (73.87%) in the OTA levels was observed, showing a synergistic effect (IR = 3.76) with respect to individual treatments.

Although there are few studies on the antitoxigenic activity of fresh and dried rosemary, its leaves have shown their capability to decrease the OTA levels produced by *P. nordicum* in dry-cured fermented sausage-based medium and in the real product (Álvarez et al., 2020; 2021a; 2022a). Furthermore, a phenolic extract from rosemary reduced the OTA generated by *A. carbonarius* in a synthetic grape medium (El Khoury et al., 2017).

On the other hand, REO has shown its ability to decrease the production of mycotoxins, such as OTA by *P. nordicum*, *A. carbonarius* and *A. westerdijkiae* (Álvarez et al., 2021a; Císarová et al., 2020; El Khoury et al., 2016), aflatoxins by *A. flavus* (Da Silva Bomfim et al., 2020) and fumonisins by *Fusarium verticillioides* (Da Silva Bomfim et al., 2015).

Despite the great reduction in the mycotoxin production, the treatment R + REO should be combined with other strategies to obtain OTA amounts complying with the limit established in the Italian regulation (Ministerio della Sanità, 1999). Other BCAs, such as *D. hansenii*, *Lactobacillus buchneri and P. chrysogenum*, could be studied in combination with R + REO due to their ability to reduce the OTA synthesis in different moulds (Delgado et al., 2019; Iacumin et al., 2020; Álvarez et al., 2021a, 2022a).

3.2. Proteomic analyses

Comparative proteomic analyses were performed to shed light on the impact of the only treatment able to reduce the OTA amount (R + REO) on the proteome of *A. westerdijkiae* and the implication of synergistic mechanisms in their effectiveness. To the best of our knowledge, this is the first proteomic approach evaluating the effect of derived herbs as BCAs on *A. westerdijkiae*. For this kind of assays, the use of a meat model under highly controlled conditions allows to identify the antifungal mechanisms of BCAs, which could be hidden in natural systems (Crowther et al., 2018).

A total of 1066 proteins were identified in the label-free quantitative (LFQ) proteomic analysis of the treatment R + REO when compared to the control (C; Supplementary Table S1). Quantitative and qualitative changes in the protein abundance were analysed (Table 1). The gene ontology enrichment analysis performed with ClueGO classified the proteins in "groups" and further subdivided them in "terms". Proteins from 22 metabolic pathways or terms were decreased, being the most of these terms grouped in the "cellular macromolecule catabolic process" (72.73%; Fig. S1). The proteins found increased belonged to 40 terms and 6 different groups. Most of the terms per group were classified in the "organic acid metabolic process" (55%; Fig. S2).

When comparing proteins from treatments R + REO and REO (Supplementary Table S2), aiming to decipher those proteins involved in the synergistic effect, the ClueGO showed proteins grouped into 10 terms reduced in abundance, being most of these terms grouped in the

Table 1
Number of proteins altered in abundance in *Aspergillus westerdijkiae* MUM 16.142 proteome in the presence of the treatment R + REO[a] when compared with its proteome in the control (C) and treatments REO and R in a dry-cured fermented sausage-based medium.

Treatment[a]	Proteins reduced in abundance	Proteins increased in abundance	Proteins only detected in the studied treatment	Proteins only detected in R + REO
C	81	114	19	39
REO	116	34	18	10
R	79	119	12	25

[a] R + REO: rosemary leaves added in the medium (R; 2 g/kg) + rosemary essential oil (REO; 100 μL on the surface of collagen casings covering the medium). C: control (without biocontrol agents).

"ribonucleoprotein complex biogenesis" (40%; Fig. S3).

The gene ontology enrichment analysis when comparing R + REO and R (Supplementary Table S3), also aiming to decipher those proteins involved in the synergistic effect, classified proteins found diminished in 12 terms belonging the 50% of the terms to the "aminoacyl-tRNA ligase activity" group (Fig. S4).

The treatment R + REO, which provoked the successful OTA repression, resulted in a drop in the abundance of some proteins associated with the mycotoxin biosynthesis in *A. westerdijkiae*, such as the PKS ER domain containing protein when compared with C (Table 2). This protein is involved in the synthesis of a polyketide synthase (PKS; Ferracin et al., 2012), indispensable for forming the ochratoxin β, an OTA precursor (Gallo et al., 2017). The deletion of a gene encoding a PKS with an ER domain in *A. ochraceus* (possibly *A. westerdijkiae*), directly abolished the mycotoxin production (Wang et al., 2015). In addition, a decrease of proteins from this domain has been related to a minor OTA production by *P. nordicum* caused by REO and *D. hansenii* as BCAs (Álvarez et al., 2021a; 2022a). This shared finding between *P. nordicum* and *A. westerdijkiae* indicates that such protein could play a key role in OTA biosynthesis regardless of the producer species.

Discriminant proteins that elucidate the synergistic effect of the successful combined treatment R + REO *versus* the inefficient individual treatments REO and R on *A. westerdijkiae* were checked. A lower abundance of PKS ER domain was found in R + REO than in the treatment with REO alone (Table 2). However, no differences between these proteins were found when R + REO and R were compared (Table 2).

Table 2
Changes in protein abundance ($p < 0.05$) for *Aspergillus westerdijkiae* MUM 16.142 in the presence of different biocontrol agents (BCAs) growing in a dry-cured fermented sausage-based medium (FS).

Treatment *vs* R + REO[a]	Proteins	Log_2 Fold change (R + REO)
C	PKS ER domain containing protein	−0.426
	Dynein light chain	−0.723
	KRE9 domain containing protein	−0.871
	Cell wall protein PhiA	1.438
REO	PKS ER domain containing protein	−0.585 and −0.674
	Dynein light chain	−0.431
	SDA1	−0.973
	Ssd1	−0.826
	SSD domain containing protein	−0.335
R	Actin binding protein Fragmin, putative	Only in R[b]
	C-24(28) sterol reductase	0.384

[a] R + REO: rosemary leaves (R) added to FS (0.2%, w/w) before plating and rosemary essential oil (REO) on the surface of collagen casings covering FS (100 μL); C: control (without BCAs).
[b] Only in R: protein detected only in the treatment R.

Therefore, no clear cumulative effect on this protein is seemingly exerted, although its involvement in the synergistic mechanism cannot be ruled out.

The cell wall integrity (CWI) was found altered in the R + REO treatment, when compared with C, by decreasing the abundance of key proteins, such as the KRE9 domain containing protein and the Dynein light chain (DLC; Table 2). To our knowledge, although there are not previous studies about the function of KRE9 in moulds, it intervenes in the synthesis of cell surface polysaccharides in yeasts, which could help the fungus to dodge the plant defences (Soanes et al., 2008). DLC is involved in the regulation of actin assembly during endocytosis and septum positioning in fungi (Lamb et al., 2021; Liu et al., 2003). Furthermore, the cell wall protein PhiA, involved in the synthesis of conidiophores, is increased in the treatment with R + REO respect to the control (Table 2). An overexpression of the *PhiA* gene has been linked to the survival response of *Aspergillus nidulans* by producing conidia in the presence of toxic metabolites (Melin et al., 2003). Thus, *A. westerdijkiae* seems to overcome the effect of the BCAs through the activation of diverse defence mechanisms at proteome level.

Alterations in the CWI were also found when R + REO was compared to REO due to the reduced abundance of the proteins DLC, SDA1, cell wall biogenesis protein phosphatase Ssd1 and the SSD domain containing protein (Table 2). Due to the absence of information about SDA1 and Ssd1 in moulds, the activity of these proteins has been compared with their functions in yeasts. The SDA1 protein is required for actin cytoskeleton organization in the yeast *Saccharomyces cerevisiae* and Ssd1 binds mRNAs encoding cell wall proteins in yeasts and represses their translation under stress conditions (Jansen et al., 2009; Thammahong et al., 2019). Proteins related to the actin cytoskeleton organization were as well altered in *P. nordicum* when using an aqueous macerate of R in dry-cured fermented sausages (Álvarez, Delgado, et al., 2022), pointing to a common repression mechanism in both moulds. This mechanism of action has been also deployed against *A. westerdijkiae* by the synergistic effect of the two BCAs. The absence of the gene that codes for Ssd1 in *Aspergillus fumigatus* reduced the colony growth and made the mould more resistant to cell-wall perturbing agents (Thammahong et al., 2019). The SSD domain containing protein is involved in the regulation of ergosterol biosynthesis (Arastehfar et al., 2021). The lower abundance of this protein is in accordance with previous findings for R and REO, which decrease the ergosterol content in other toxigenic moulds such as *P. nordicum*, *A. flavus* and *F. verticillioides* (Da Silva Bomfim et al., 2015, 2020; Álvarez et al., 2021b).

The proteomic analysis of the treatment R + REO *versus* R showed that the CWI was altered by the decrease in abundance of the protein actin binding protein Fragmin, putative (Table 2), linked to actin filament, when R + REO was applied. Considering these results, different proteins related to the actin binding and cytoskeleton organization could contribute to the synergistic effect of R + REO, being the actin one of the antifungal targets of such combined treatment. On the contrary, the C-24(28) sterol reductase, a protein directly involved in ergosterol biosynthesis pathway, was increased.

Regarding proteins associated with the ergosterol synthesis, the combined treatment R + REO provoked a decrease in the abundance of the SSD domain with respect to REO, but an increase in C-24 with respect to R. Thus, these differences could explain why the ergosterol synthesis pathway is not altered in the treatment R + REO compared to the control.

Considering all the results, the antiochratoxigenic effect of the combination R + REO might be mainly due to its action on PKS ER domain containing protein and, to a lesser extent, to its effects on CWI related proteins. This synergy seems to lead to the decrease in the abundance of PKS ER domain containing protein when compared with the isolated effect of the REO treatment. However, the role of R in the synergistic effect needs further research to be clearly elucidated. The alteration observed in these pathways by the effect of BCAs can be used to set the molecular bases of antifungal targets to enhance additive and

synergistic effects with other different BCAs.

4. Conclusions

The treatment R + REO resulted in a dramatic OTA reduction, up to 73.87%, although the OTA levels found are still well over the Italian legislation limit. REO alone exerts similar effect on the mould proteome than its combination with R, but in a lesser extent. However, the impact of R in the *A. westerdijkiae* proteome with respect to its combination with REO is not conclusive. Thus, the combination of these two BCAs along with other strategies to further reduce the OTA production could be set within the HACCP framework to minimise the hazard due to ochratoxigenic *A. westerdijkiae* in dry-cured fermented sausages.

Funding

This research was funded by Foundation for Science and Technology (FCT, Portugal) through financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020). Junta de Extremadura-Consejería de Economía, Ciencia y Agenda Digital-, Fondo Europeo de Desarrollo Regional-"Una manera de hacer Europa" (grant number GR18056), and grant PID2019-104260GB-I00 funded by MCIN/AEI/10.13039/501100011033. M. Álvarez is recipient of the grant BES-2017-081340 funded by MCIN/AEI/10.13039/501100011033 and by "ESF Investing in your future". Q-Exactive mass spectrometer to proteomic research was acquired by the grant UNEX-AE-3394 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe"

CRediT authorship contribution statement

Micaela Álvarez: Formal analysis, Investigation, Methodology, Validation, Data curation, Visualization, Writing – original draft. **María J. Andrade:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Josué Delgado:** Methodology, Data curation, Supervision, Software, Writing – review & editing. **Félix Núñez:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Ángel-Carlos Román:** Data curation, Software. **Paula Rodrigues:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The obtained data can be found in the manuscript and the supplementary material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.109436.

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