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Original article

***Penicillium* species during the manufacturing and ripening of Armada raw goat's milk cheese: identification, characteristics and in vitro potential toxins production**

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**Abstract** — The species and characteristics of the moulds of the *Penicillium* genus were studied throughout the manufacturing and ripening of Armada cheese. Along the ripening of four batches of cheese, samples of milk, curd, and 1-, 2-, 4-, 8-, and 16-week-old cheese were studied. Of the 274 isolates obtained from the Oxytetracycline Glucose Yeast-Extract Agar medium throughout the manufacturing and ripening, 28 were considered moulds belonging to the *Penicillium* genus and were identified using both morphological and physiological tests as following: *Penicillium roqueforti* (10 strains), *P. crustosum* (6 strains), *P. commune* (4 strains), *P. aurantiogriseum* (3 strains), *P. verrucosum* chemotype I (2 strains), and *P. chrysogenum* (3 strains). The presence of the species of the *Penicillium* genus was more important in the last stages of ripening. This fact could be related to the marked increase in the content of total free fatty acids, and of short-chain fatty acids in particular, which is observed after the second month of ripening in this cheese. The mycotoxin production was investigated by thin-layer chromatography techniques. All the strains were toxicogenic in vitro. © Inra/Elsevier, Paris.

**Armada goat cheese / mould / *Penicillium* / mycotoxin**

**Résumé** — Étude des espèces du genre *Penicillium* pendant la fabrication et l'affinage du fromage de chèvre au lait cru de la variété Armada. Identification, caractéristiques et capacité de production de toxines in vitro. Nous avons étudié les espèces et les caractéristiques des moisissures du genre *Penicillium* pendant l'élaboration et la maturation de quatre fabrications de fromage Armada.

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Dans les quatre fabrications, des échantillons de lait, de caillé et de fromage âgé de 1, 2, 4, 8, et 16 semaines ont été analysés. Des 274 souches isolées à partir du milieu OGYEA, 28 ont été identifiées comme moisissures appartenant au genre *Penicillium*. Elles se répartissent ainsi : *Penicillium roqueforti* (10 souches), *P. crustosum* (6 souches), *P. commune* (4 souches), *P. aurantiogriseum* (3 souches), *P. verrucosum* chimiotype I (2 souches) et *P. chrysogenum* (3 souches). La présence d'espèces du genre *Penicillium* est plus importante à la fin du processus d'affinage, ce qui semble être en rapport avec la forte augmentation de la teneur en acides gras totaux, en particulier à courte chaîne, que l'on observe dans ce fromage à partir du deuxième mois d'affinage. La production de mycotoxines a été mise en évidence par chromatographie sur couche mince. In vitro, toutes les souches se révélaient toxigènes. © Inra/Elsevier, Paris.

## fromage de chèvre Armada / moisissure / *Penicillium* / mycotoxine

### 1. INTRODUCTION

Moulds are very ubiquitous microorganisms which are found in the majority of food. They have a high metabolic activity and can produce positive and negative effects.

Moulds are used as starter cultures in the elaboration of certain food such as cheese, other dairy products, sausages and cured ham. A lot of cheeses are ripened by moulds, with the species of the genus *Penicillium* (*P. roqueforti*, *P. camemberti*, *P. nalgiovense* and *P. chrysogenum*) being the most used species [20]. The proteolytic and lipolytic enzymes of moulds participate markedly in the ripening of a certain number of cheeses. Proteolysis is needed for the development of an adequate texture and the characteristic flavour. The resulting amino acids are forerunners of aldehydes, alcohols and esters. They can, at the same time, produce desaminations of amino acids; the free ammonia equally contributes to the formation of the aroma. The lipolytic activity of the moulds is also important in the ripening of certain types of cheeses. The free fatty acids generated in the degradation process of the lipids have their own taste. Moulds are also capable of oxidizing fatty acids to methyl ketones and of producing from these secondary alcohols.

Moulds are also important as they are responsible for undesirable effects in cheese.

They are one of the main causes of spoilage in cheese, provoking musty flavours and an unsightly appearance [1].

Furthermore, some moulds are also able to produce mycotoxins in the cheese dangerous for the consumer. As starter cultures, those strains which do not produce mycotoxins, at least the known ones, are preferred as some of them are highly toxic.

This work aims at the study of species and characteristics of moulds of the *Penicillium* genus present in Armada cheese, a variety of Spanish goat's cheese made from raw milk, the role that these moulds plays in the ripening as well as their capability of producing mycotoxins in synthetic culture media since this criterion is taken into account when selecting the strains used as starter cultures.

### 2. MATERIALS AND METHODS

#### 2.1. Cheese samples

Four batches of Armada cheese were elaborated by experienced cheesemakers following the traditional methods as described by Tornadijo et al. [31]. From each batch, milk, curd, and 1-, 2-, 4-, 8- and 16-week-old cheese samples were taken. Each cheese sample was made up of one whole cheese. Samples were transported to the laboratory under refrigeration (below 5 °C) and analyzed on arrival.

## 2.2. Microbiological analysis

Fifty g of each sample (after discarding the rind of the cheeses) were homogenized with 200 mL of a sterile solution of 2% sodium citrate at 40–45 °C for 1 min in a Stomacher 400 Lab Blender (Seward Medical, London, England), thus making a 1/5 dilution. Consecutive decimal dilutions were prepared by mixing 10 mL of the previous dilution with 90 mL of 0.1% sterile peptone water. One mL of each dilution was inoculated in duplicate in 20 mL of Oxytetracycline Glucose Yeast-Extract Agar (Oxoid) [22] and mixed before solidification. Plates were incubated for 5 days at 22 °C.

## 2.3. Isolation and identification of strains

From the plates with 30 to 300 colonies, 10 random colonies were taken from each sampling point with aid of a Harrison disc [15]. The 274 isolates obtained were purified and maintained in Sabouraud dextrose agar (Oxoid) tubes under 4 °C covered with sterile parafin. From the 274 isolates obtained, 28 were considered moulds of the *Penicillium* genus. Moulds were firstly differentiated from yeasts with regard to morphology and type of growth. The moulds were grown in three different media: Czapek agar, Czapek yeast autolysate extract agar (CYA) and Malt extract agar (MEA). In each one of these media the moulds acquired a characteristic morphology. After 7 d of incubation at 25 °C, each of the cultures were macroscopically examined and the diameter of the colonies, the colour, the texture, the pigment production and the exudate production were noted.

For the microscopic examination, preparations were carried out by taking a portion of the mycelium with a sterile needle and using lactophenol, lactophenol-picric acid and lactophenol-blue cotton as colourants.

### 2.3.1. Identification at genus level

In order to identify the isolates at genus level, the Samson and van Reenen-Hoekstra [29] methods were followed. The following characteristics were observed in each isolate: the presence of septed mycelia and the production of asexual spores within sporangium or from hypha, the presence of fruit-bodies, the way of conidia formation (from conidiogenous cells or by fragmentations of the hyphae), the disposition of the conidiophore and the form of the conidia.

### 2.3.2. Identification at species level

The identification at species level of the moulds of the genus *Penicillium* was carried out by examining the morphological and physiological characteristics. The study of the macro and microscopic morphological characteristics allowed for a previous identification at species level following the methods of Raper and Thom [27], Ramírez [26], Pitt [25] and Samson and van Reenen-Hoekstra [29]. This identification was later confirmed using physiological tests. After incubation at 25 °C for 7 d in the Czapek agar, CYA and MEA media, macroscopic characteristics were observed (colour; texture (velvetlike, fasciculate, woollike, funiculose); smell; exudate production; pigment production; diameter of the colonies) and microscopic characteristics (structure of the conidiophore (grade of ramification between phialide and stipe); form and dimensions of the phialide, metula and rami; surface of the stipe (smooth, rough, verrucose); and size, surface and colour of the conidia.

In the genus *Penicillium* some isolates are usually difficult to identify only with regard to morphological characteristics as some criteria such as the texture and the colour are fairly subjective. Some species are also difficult to identify observing their microscopic characteristics. For a better identification, in agreement with the criteria of Frisvad [10] and Pitt [25], the following physiological characteristics were studied: reaction with FeCl<sub>3</sub>; ability to produce rottenness in apples and/or in citric fruits; milk digestion; growth in creatine-sucrose agar (CREA); growth in agar and in solution with NO<sub>2</sub><sup>-</sup>; growth at 5, 30, 34 and 37 °C; inhibition of growth of *Staphylococcus aureus*, *Candida spp.*, *Bacillus cereus* and *Bacillus subtilis*; lipolytic activity (growth on tricaproin and tributyrin agar); growth in presence of fungicides using the following culture media: GYBS (Glucose Yeast extract agar with 50 ppm of sorbic acid and 50 ppm of benzoic acid), GYP (GY agar with 1 000 ppm of propionic acid), and GYA (GY agar with 5 000 ppm of acetic acid).

## 2.4. Production of mycotoxins by the *Penicillium* species

The strains of the genus *Penicillium* were tested for the production of mycotoxins since those strains which do not produce any known toxins are preferred as starter cultures. Moreover, accord-

ing to Frisvad and Filtenborg [11] the terverticillate *Penicillia* can be defined according to their secondary metabolite profiles as they can be used as a taxonomic criterium. The strains were grown in different media (CYA, OA and YES) at 25 °C for 7–14 d. In order to produce the mycotoxins, two techniques were followed: the one of toxins extraction [10] and the one of the agar top [6, 7]. The identification of the mycotoxins was carried out by thin-layer chromatography techniques using TLC aluminium plates silica gel 60 WF<sub>264</sub>s from Merck and specific revelations. Griseofulvin, mycophenolic acid, penicillic acid, roquefortine and penitrem A were used as standards at 0.5 mg·mL<sup>-1</sup>. Some tests were carried out by previously impregnating the plates in oxalic acid at 8 % in water or in methanol, air-drying them afterwards; better results were obtained with the acid mycotoxins using this method.

The mixtures TEF (toluene/ethylacetate/90 % formic acid (5:4:1)) and CAP (chloroform/acetone/propane-2-ol [85:15:20]) were used as eluants. Once the runs had finished, the plates (before and after the treatment with different reagents) were observed under visible and ultraviolet at 254 nm lights; some mycotoxins were visible without any type of treatment. The plates were developed with chemical reagents pulverization or exposition to their vapours. Mycotoxins appeared as coloured or fluorescent spots [7, 10, 11, 29]. The used reagents with the plates eluted with TEF and the mycotoxins which were visualized with each one of them were the following:

- reagent 1 (R<sub>1</sub>): 1 % FeCl<sub>3</sub> (w/v) in butane-1-ol. It allowed us to visualize griseofulvin, mycophenolic acid, PR-toxin, roquefortine C, and ochratoxin A;

- reagent 2 (R<sub>2</sub>): 50 % H<sub>2</sub>SO<sub>4</sub> in water, heating for 5 min at 120–130 °C; it allowed us to visualize griseofulvin, penicillic acid, mycophenolic acid, PR-toxin, penitrem A, rugulovasine, cyclopiazonic acid, and cyclophenol/cyclophenin;

- reagent 3 (R<sub>3</sub>): ANIS (0.5% p-anisaldehyde in methanol or in ethanol/acetate acid/concentrated sulphuric acid [17:2:1]), heating the plate for 5 min at 120 °C; it allowed us to visualize above all griseofulvin, mycophenolic acid, penicillic acid, penitrem A, roquefortine C, and terrestic acid;

- reagent 4 (R<sub>4</sub>): exposure to NH<sub>3</sub> vapours for 1–3 min; after this, plates were pulverized with ANIS and heated; it allowed us to visualize griseofulvine and penicillic acid;

- reagent 5 (R<sub>5</sub>): 20 % AlCl<sub>3</sub> (w/v) in 96 % ethanol, the plates were then heated at 120 °C for 5 min. It allowed us to visualize penitrem A;

- reagent 6 (R<sub>6</sub>): 1 % FeCl<sub>3</sub> (w/v) in butane-1-ol and ANIS heating the plates at 120 °C for 10 min. It allowed us to visualize griseofulvin, penitrem A, roquefortine C, rugulovasine, and cyclopiazonic acid.

For the plates eluted with CAP the reagent 7 (R<sub>7</sub>) was used: 1 % Ce(SO<sub>4</sub>)<sub>2</sub> in H<sub>2</sub>SO<sub>4</sub> 6N. It allowed us to visualize roquefortine C, griseofulvin, penitrem A, mycophenolic acid, penicillic acid, rugulovasine, and cyclophenol/cyclophenin.

To identify the mycotoxins the absolute Rf and the relative Rf of griseofulvin (Rfg) were measured.

### 3. RESULTS

Of the 274 strains obtained from the OGYEA medium during the manufacturing and ripening of the four batches of Armada cheese, twenty eight were appointed to the genus *Penicillium*. Of the characteristics which are shown, a green or white mycelium with a good sporulation, conidiophores in an artist's brush form (*penicillium*), presence of conidiogenous cells (phialides), metulae and rami stand out. The phialides are bottle-shaped or, if longer, spear-shaped. The conidia come from these through basipetal succession.

The identification at the species level of the 28 strains of the genus *Penicillium* was as following.

Ten strains were identified as *P. roqueforti*; the identification of these strains did not pose any great difficulty since the morphology both macro and microscopically is very characteristic. Three strains were identified as *P. chrysogenum*, and six as *P. crustosum*. Nine strains were included in the group *P. verrucosum* complex according to Samson and van Reenen-Hoekstra [29]; within this group paying attention above all to the physiological characteristics 2 strains were identified as *P. verrucosum* chemotype I, 4 as *P. commune*, and 3 as *P. aurantiogriseum*.



Table I shows the distribution of these species at the different sampling points and their morphological characteristics. Their physiological and cultural characteristics are shown in table II.

Table III shows the mycotoxins produced in vitro by the strains of the *Penicillium* genus isolated, their Rfg values using TEF and CAP as eluants and the reagents used in the developing. The 10 *P. roqueforti* strains produced roquefortine C, mycophenolic acid, penicillic acid and PR-toxin. The 3 *P. chrysogenum* strains produced roquefortine C and PR-toxin. Of the 6 *P. crustosum* strains, 1 produced penitrem A, terrestrial acid, roquefortine C, and rugulovasine; 3 produced penitrem A, terrestrial acid, and roquefortine C; 1 produced roquefortine C, penitrem A, and rugulovasine; the remaining strain produced penitrem A and terrestrial acid. The 2 *P. verrucosum* strains produced ochratoxin A, this mycotoxin is exclusively produced by *P. verrucosum* and thus makes up a differential character of this species. Of the 4 *P. commune* strains, 1 produced rugulovasine, penitrem A, cyclophenol/cyclophenin, and cyclopiazonic acid; 1 produced rugulovasine, penitrem A, and cyclophenol/cyclophenin; 1 produced rugulovasine and penitrem A; the remaining strain produced cyclopiazonic acid and cyclophenol/cyclophenin. Lastly, of the 3 *P. aurantiogriseum* strains, 1 produced terrestrial acid, rugulovasine and penitrem A; 1 produced terrestrial acid, rugulovasine and cyclopiazonic acid; the remaining strain produced terrestrial acid and penitrem A.

#### 4. DISCUSSION

The criteria used for the identification of the isolated moulds based both on the morphological and physiological characteristics can be justified because although the taxonomy of the moulds traditionally has been carried out with almost exclusive regard to morphological criteria, both macroscopic and microscopic, within the *Penicil-*

*lia terverticillata* there exists a great variability which is seen both in macromorphology and in micromorphology; successive cultures of the same strain in the same medium can give rise to colonies of different dimensions and aspects.

The texture of the colonies was one of the criterion used by Raper and Thom [27] to establish taxons in the genus *Penicillium*. This criterion as well as being artificial is very subjective as Ciegler et al. [2] have already established which is why it is very difficult to situate a species into one or another section. Even Raper and Thom [27] considered intergradations exist between sections, in such a way that a same species could have variations in some characteristics; successive cultures of the same strain could also affect the morphological characteristics of the colonies. Other authors such as Ramírez [26] and Pitt [25] used similar criteria, although Pitt [25] placed more emphasis on the micromorphological characters. Either way important micromorphological differences can not always be observed, as certain asymmetric *Penicillia* may have one or more sized branches which end in verticiles of metula and phialides, and conidiophores more or less long which can be smooth, rough or slightly rough [2]. Therefore, complementary tests are needed at least to confirm identification, above all within the group that Samson and van Reenen-Hoekstra [29] described as *P. verrucosum* complex. Many species included in this group were the object of frequent taxonomic confusions [11]. Various authors have begun to establish physiological criteria which could be applied to the taxonomy of moulds. Samson and van Reenen-Hoekstra [29] briefly included this possibility, however there is a lack of available information in order to establish a relationship between the physiological characters and taxons.

With regard to the *Penicillium* species found throughout the making and ripening of Armada cheese predominated *P. roqueforti*

**Table I.** Morphological characteristics\* and distribution in the sampling points of *Penicillium* species isolated during the manufacturing and ripening of Armada cheese (four batches).**Tableau I.** Caractères morphologiques\* et distribution aux stades d'échantillonnage des souches de moisissures du genre *Penicillium* isolées durant la fabrication et l'affinage du fromage Armada (quatre fabrications).

|                                     | <i>P. roqueforti</i>            | <i>P. chrysogenum</i>                                | <i>P. crustosum</i>                       | <i>P. verrucosum</i><br>chemotype I   | <i>P. commune</i>                         | <i>P. aurantiogriseum</i>                 |
|-------------------------------------|---------------------------------|--|---|---------------------------------------|---|---|
| No. of isolates                     | 10                              | 3  | 6   | 2                                     | 4   | 3   |
| Distribution in the sampling points | milk (2)<br>16-w-old cheese (8) | curd (1)<br>2-w-old cheese (1)<br>8-w-old cheese (1) | 8-w-old cheese (1)<br>16-w-old cheese (5) | curd (1)<br>8-w-old cheese (1)        | 4-w-old cheese (1)<br>16-w-old cheese (3) | 2-w-old cheese (1)<br>16-w-old cheese (2) |
| Smell                               | Not detectable                  | Slight (aromatic)                                    | Noticeable (fruity)                       | Strong (glue or acetone)              | Strong (mouldy) in old cultures           | Noticeable (fruity)                       |
| Exudate                             | No                              | Abundant (yellowish)                                 | Abundant (colourless drops)               | Slight (bright drops)                 | Slight                                    | Slight (colourless drops)                 |
| Colour in MEA                       | Green-grey                      | Green-grey   | Green-grey                                | Green (white edges)                   | Green                                     | Green (white edges)                       |
| Diameter in MEA                     | 7 cm                            | 2.4–2.5 cm   | 2.5 cm                                    | 1.1–1.2 cm                            | —   | 1 cm                                      |
| Texture in MEA                      | Velvet-like                     | Velvet-like  | Very sporulated                           | Cotton-like                           | —   | Velvet-like                               |
| Aspect in MEA                       | Spider's web                    | Flat   | They easily detach masses of conidia      | Central area with a dark green colour | —   | Not flat                                  |
| Colour in Czapek                    | Green-greyish                   | Green-grey   | Greyish-green                             | White                                 | White (green centre)                      | White                                     |
| Diameter in Czapek                  | 1.5–2 cm                        | —  | 2.5 cm                                    | 1.1 cm                                | 1.5 cm                                    | 1 cm                                      |
| Texture in Czapek                   | —                               | Velvet-like  | —   | Cotton-like                           | —   | Cotton-like                               |
| Colour in CYA                       | Green-grey (white edge)         | Green or yellowish green (white edges)               | Green (white edge)                        | White                                 | Green-blue                                | Green (white edge)                        |
| Diameter in CYA                     | —                               | 3.5–3.7 cm   | 3 cm                                      | 1.5 cm                                | 2–3.5 cm                                  | 1.5 cm                                    |
| Aspect in CYA                       | Flat with a higher central area | Radially grooved                                     | Radially grooved                          | Radially grooved                      | Radially grooved                          | Radially grooved                          |
| Reverse side                        | Dark green (almost black)       | Yellow green or pale yellow                          | Yellow-cream                              | Brown-cinnamon halos                  | Creamy white                              | Bright yellow-orange                      |

Table I. (Continued). Tableau I. (Suite).

|                     | <i>P. roqueforti</i> | <i>P. chrysogenum</i>           | <i>P. crustosum</i>                   | <i>P. verrucosum</i><br>chemotype I | <i>P. commune</i>         | <i>P. aurantiogriseum</i> |
|---------------------|----------------------|---------------------------------|---------------------------------------|-------------------------------------|---------------------------|---------------------------|
| Conidiophores       | Tertverticillate     | Bi and tertverticillate         | Bi and tertverticillate               | Ter and quater-<br>verticillate     | Bi and tertverticillate   | Bi and verticillate       |
| Stipes              | Warty                | Smooth or slightly<br>rough     | Smooth or<br>slightly rough           | Finely spinulose                    | Smooth or<br>finely rough | Smooth or very            |
| Conidia             | Fairly large         | Elliptic became<br>more globose | Elliptical, globose<br>and subglobose | Globose or subglobose               | Globose or subglobose     | —                         |
| Diameter of conidia | Until 6 µm           | 3-4 µm                          | 4 µm                                  | 2-3 µm                              | 3-5 µm                    | —                         |

\* After incubation 7 d at 25 °C.

\* Après incubation de 7 j à 25 °C.

which was isolated from milk and from sixteen-week-old cheese. Obviously, the levels of *P. roqueforti* found in Armada cheese are much lower than those observed in blue-veined cheeses [3, 5, 12, 24]. The presence of this species in the last stages of the ripening of Armada cheese may be explained by the presence of cracks in some of the cheeses which ease its implantation in the inside of the mass.

*P. roqueforti* had an intense proteolytic activity [13], which does not fully agree with the low levels of proteolysis observed in Armada cheese [9]. Possibly the high values of salt/moisture ratios in Armada cheese [8] are responsible to the low activity of the *P. roqueforti* enzymes in this cheese; it is known that the activity of *P. roqueforti* proteases falls as the concentration salt/moisture rises to above 0.5 [18]. On the other hand, a great variability in the proteolytic activity of the *P. roqueforti* strains has been observed, showing that, in general, the strains with a high proteolytic capacity have a low lipolytic capacity and reciprocally [23, 28]. Given the intense lipolysis undergone by Armada cheese [9] and the possible participation of *P. roqueforti* in this process in the last stages of ripening, it is possible that the *P. roqueforti* strains in this cheese have a weak proteolytic activity. The proteolytic capacity of the *Penicillium* species isolated from Armada cheese also could be inhibited by high concentrations of free fatty acids generated during ripening [16].

With regard to the possible participation of *P. roqueforti*, and that of the other species of the *Penicillium* genus isolated, in the lipolytic processes that take place during the ripening of Armada cheese, the presence of these species could be related to the marked increase in the content of total free fatty acids, and of short-chain fatty acids in particular, which is observed after the second month of ripening [9]. The *P. roqueforti* lipases have a very intense activity, characterized by a high production of caprylic acid and other short-chain fatty acids [14]. More-



**Table II.** Physiological characteristics of the *Penicillium* strains isolated throughout the manufacturing and ripening of Armada cheese.**Tableau II.** Caractères physiologiques des souches du genre *Penicillium* isolées au cours de la fabrication et de l'affinage du fromage Armada.

|   | <i>P. roqueforti</i> | <i>P. chrysogenum</i> | <i>P. crustosum</i> | <i>P. verrucosum</i><br>chemotype I | <i>P. commune</i> | <i>P. aurantiogriseum</i> |
|---|----------------------|-----------------------|---------------------|-------------------------------------|-------------------|---------------------------|
| No. of strains                              | 10                   | 3                     | 6                   | 2                                   | 4                 | 3                         |
| Rottenness in apples                        | 0                    | 0                     | 6                   | 0                                   | 2                 | 0                         |
| Rottenness in citric fruits                 | 10                   | 2                     | 0                   | 1                                   | 1                 | 1                         |
| Milk coagulation                            | 0                    | 3                     | 0                   | 0                                   | 0                 | 0                         |
| Growth in:                                  |                      |                       |                     |                                     |                   |                           |
| Tricaproin agar                             | 0                    | 0                     | 0                   | 0                                   | 0                 | 0                         |
| Tributyryn agar                             | 10                   | 3                     | 6                   | 2                                   | 4                 | 3                         |
| Growth at 5 °C                              | 0                    | 0                     | 0                   | 0                                   | 0                 | 0                         |
| Growth at 30 °C                             | N.D.                 | N.D.                  | 0                   | N.D.                                | 0                 | 0                         |
| Growth at 34 °C                             | N.D.                 | N.D.                  | 0                   | N.D.                                | 0                 | 0                         |
| Growth at 37 °C                             | 0                    | 0                     | 0                   | 0                                   | 0                 | 0                         |
| Reaction with FeCl <sub>3</sub>             | 10 yellow            | 3 orange              | 3 yellow, 3 orange  | 1 yellow, 1 orange                  | 2 yellow          | 0                         |
| Inhibition of:                              |                      |                       |                     |                                     |                   |                           |
| <i>Candida</i> spp.                         | 0                    | 0                     | 0                   | 0                                   | 0                 | 3                         |
| <i>S. aureus</i>                            | 0                    | 3                     | 4                   | 1                                   | 0                 | 2                         |
| <i>B. cereus</i>                            | 0                    | 3                     | 5                   | 0                                   | 3                 | 2                         |
| <i>B. subtilis</i>                          | 10                   | 3                     | 5                   | 1                                   | 3                 | 2                         |
| Growth in CREA                              | 10                   | 3                     | 6                   | 0                                   | 4                 | 0                         |
| Acid in CREA                                | 0                    | 3                     | 6                   | 0                                   | 4                 | 3                         |
| Alkali in CREA                              | 0                    | 0                     | 6                   | 0                                   | 4                 | 0                         |
| Growth in agar NO <sub>2</sub> <sup>-</sup> | 10                   | 0                     | 6                   | 2                                   | 0                 | 0                         |
| Caseinolytic activity                       | 10                   | 3                     | 6                   | 2                                   | 3                 | 3                         |
| Growth in:                                  |                      |                       |                     |                                     |                   |                           |
| GYBS  | 10                   | 3                     | 6                   | 0                                   | 4                 | 0                         |
| GYP   | 10                   | 0                     | 6                   | 0                                   | 2                 | 0                         |
| GYA   | 10                   | 0                     | 0                   | 0                                   | 0                 | 0                         |

N.D : not determined.

N.D. : non déterminé.

**Table III.** Relative Rf values to griseofulvin (Rfg)\*, visualization and colours of the mycotoxins produced in vitro by the *Penicillium* strains isolated throughout the manufacturing and ripening of the Armada cheese.

**Tableau III.** Rf relatif par rapport à la griseofulvine, visualisation et couleurs des mycotoxines produites in vitro par les souches du genre *Penicillium* isolées durant la fabrication et l'affinage du fromage Armada.

| Species                             | Rfg in TEF                 | Mycotoxins        | Developing                 | Rfg in CAP  | Developing        | No. of producing strains |
|-------------------------------------|----------------------------|-------------------|----------------------------|-------------|-------------------|--------------------------|
| <i>P. roqueforti</i>                | 0.07 – 0.08                | Roquefortine C    | none<br>R6 – visible       | 0.22 - 0.21 | R7 – red          | 10/10                    |
|                                     | 1.26 – 1.34                | Mycophenolic acid | none<br>R2 – yellow        | 1.04 – 1.03 | none              | 10/10                    |
|                                     | 1.05 – 1.08                | Penicillic acid   | R2 – yellow                | 0.83 – 0.88 | R7 – blue         | 10/10                    |
|                                     | 1.41                       | PR – toxin        | R1                         |             |                   | 10/10                    |
| <i>P. chrysogenum</i>               | 0.11 – 0.14                | Roquefortine C    | R6 – orange<br>R1 – brown  | 0.43 – 0.47 | R7 – blue – green | 3/3                      |
|                                     | 1.39 – 1.43                | PR – toxin        | none<br>R2 – dark          | 1.11 – 1.13 | none              | 3/3                      |
| <i>P. crustosum</i>                 | 0.57 – 0.64                | Terrestric acid   | none                       |             |                   | 3/6                      |
|                                     | 0.72 – 0.80                |                   | R3 – yellow                |             |                   | 5/6                      |
|                                     | 0.05 – 0.06                | Rugulovasine      | R6 – dark                  |             |                   | 2/6                      |
|                                     | 0.07 – 0.14                | Roquefortine C    | R3 – violet                | 0.41 – 0.42 | none              | 5/6                      |
|                                     | 1.51 – 1.55<br>1.56 – 1.74 | Penitrem A        | R2 – orange<br>R3 – violet | 1.12 – 1.22 | R7 – dark         | 6/6                      |
| <i>P. verrucosum</i><br>chemotype I | 1.35 – 1.39                | Ochratoxin A      | none<br>R1 – blue          | 0.31        | none              | 2/2                      |

**Table III. (continued).** Relative Rf values to griseofulvin (Rfg)\*, visualization and colours of the mycotoxins produced in vitro by the *Penicillium* strains isolated throughout the manufacturing and ripening of the Armada cheese.

**Tableau III. (suite).** Rf relatif par rapport à la griseofulvine, visualisation et couleurs des mycotoxines produites in vitro par les souches du genre *Penicillium* isolées durant la fabrication et l'affinage du fromage Armada.

| Species                   | Rfg in TEF                 | Mycotoxins                  | Developing                     | Rfg in CAP  | Developing       | No. of producing strains |
|---------------------------|----------------------------|-----------------------------|--------------------------------|-------------|------------------|--------------------------|
| <i>P. commune</i>         | 0.05 – 0.08                | Rugulovasine                | R6 – dark<br>R2 – blue         | 0.08        | R7 – blue<br>2/3 | 1/3                      |
|                           | 1.51 – 1.60<br>1.55 – 1.70 | Penitrem A                  | R2 – orange<br>R3 – violet     | 1.09 – 1.14 | none             | 3/3                      |
|                           | 1.30 – 1.39                | Cyclopiazonic acid          | none<br>R2 – blue<br>R6 – blue |             |                  | 2/3                      |
|                           | 1.03 – 1.05                | Cyclophenol/Cyclo-<br>penin | none<br>Oxalic – R2 – blue     | 0.84 – 0.92 | R7 – blue        | 3/3                      |
| <i>P. aurantiogriseum</i> | 1.51– 1.55                 | Penitrem A                  | R2 – orange<br>R3 – violet     | 1.09– 1.13  | none             | 2/3                      |
|                           | 0.58 – 0.69                | Terrestic acid              | none                           |             |                  | 3/3                      |
|                           | 1.39                       | Cyclopiazonic acid          | none                           |             |                  | 1/3                      |
|                           | 0.05 – 0.06                | Rugulovasine                | R6– dark                       |             |                  | 2/3                      |

\* Rf values of griseofulvin were 0.36–0.38 in TEF and 0.65–0.70 in CAP. Eluants: TEF: toluene/ethylacetate/90% formic acid (5:4:1). CAP: chloroform/acetone/propane-2-ol (85:15:20).

\* Les valeurs du Rf de la griseofulvine furent 0,36–0,38 avec TEF et 0,65–0,70 avec CAP.

over, this activity is not seen to be strongly inhibited by low pH values above 5 [30] or by high salt/moisture concentrations above 8% [17].

All the isolated *Penicillium* strains produced in vitro any mycotoxin. Our results corroborate the findings of other authors; according to information reported by Leistner [20], 75% of the *Penicillium* strains isolated from foods and the majority of the *Penicillium roqueforti* strains produce some mycotoxin.

However, the capacity to produce mycotoxins is very different in cheese and in synthetic culture media. Eventhough moulds find a good medium of growth in cheese, it does not seem adequate for the production of mycotoxins due its chemical composition [4]. Some mycotoxins are produced in low quantities or they either they become unstable in cheese, thus reducing their biological activity. Some authors associate this fact with the high protein content in cheese [21], the toxins can combine with amino acids and other compounds (-SH radicals, ammonia) and thus become inactive [19]. The production of mycotoxins can also be influenced by the Aw and the temperature of the medium [19]. The Aw values of cheese are not adequate for the production of these metabolites, nor its temperature since cheeses before being sold are usually kept in refrigeration.

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