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Original article

Composition and physical properties of the *Penicillium camemberti* mycelium

C Bizet, S Desobry *, J Fanni, J Hardy

Laboratoire de physico-chimie et génie alimentaires, École nationale supérieure d'agronomie et des industries alimentaires, Institut national polytechnique de Lorraine, 2, avenue de la Forêt-de-Haye, 54500 Vandœuvre-lès-Nancy, France

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Summary — This paper concerns the *Penicillium camemberti* (PC) mycelium that grows on French soft cheeses, like Camembert or Brie. According to our knowledge, no author considered the PC as a packaging film around the cheese. Indeed, separation of PC and cheese is not possible because PC is rooted in the cheese crust. In the present study, we grew PC on agar gel and it could easily be separated from the medium. Chemical composition and physical properties of the film were determined using raw or laminated mycelium films. The raw PC mycelium simulated the film during industrial process before packaging and the laminated film simulated the film compressed in the packaging. PC mycelium had a high elasticity allowing its adaptation to cheese-dimensional changes during industrial processing. The water vapor permeability of 2.42 10^{-10} kg/m/s/Pa and a high capacity for water vapor fixation limit water transport from cheese crust to the atmosphere. PC mycelium acts as the first packaging film around cheese.

mycelium composition / mycelium properties / *Penicillium camemberti*

Résumé — Composition et propriétés physiques du mycélium de *Penicillium camemberti*. Cet article concerne le *Penicillium camemberti* (PC) que l'on trouve à la surface des fromages à pâte molle et à croûte fleurie blanche de type camembert ou brie. Nous n'avons pas connaissance d'article de la littérature concernant l'étude des propriétés de PC en tant que premier film d'emballage autour du fromage. Cela découle essentiellement de la difficulté à séparer le mycélium du fromage pour l'étudier. En effet, le mycélium est enraciné dans la croûte du fromage et il est impossible de le séparer sans l'altérer. Dans cet article, PC se développe sur un milieu à base d'agar et de composition nutritive proche de celle du fromage. Cela a permis de séparer très facilement le mycélium du milieu, afin de l'étudier sous deux formes : brute ou laminée. La forme brute simule le mycélium en surface du fromage dans la fromagerie. La forme laminée simule le mycélium tassé entre le fromage et l'emballage, après conditionnement. La composition du mycélium de PC a ainsi été établie. Du point de

* Correspondence and reprints

vue des caractéristiques physiques, le mycélium présente une grande élasticité, qui lui permet de s'adapter à la déformation durant le conditionnement du fromage. La perméabilité à la vapeur d'eau, égale à 2,42 10^{-10} kg/m/s/Pa et la forte capacité de fixation de vapeur d'eau du film ralentissent les transferts d'eau entre la croûte du fromage et l'air ambiant. Le PC joue effectivement le rôle de premier emballage autour du fromage.

composition du mycélium / propriété du mycélium / *Penicillium camemberti*

INTRODUCTION

Penicillium camemberti (PC) is the surface mould involved in the ripening process of soft cheeses like Camembert or Brie. PC mycelium is partly responsible for the rheological and sensorial properties. According to our knowledge, ripening has been studied extensively but water or gas transfer from cheese to atmosphere through *Penicillium* has not been investigated. In a previous paper, Desobry and Hardy (1994) modeled water transport from cheese center to surrounding air, neglecting the possible limiting influence of PC mycelium. Indeed, it was not possible to separate the PC mycelium from the cheese without alternating the film. The French patent n° 2 721 040 presented a specific medium on which PC mycelium can be cultivated and separated from the medium to get a free *Penicillium* sheet (Bizet et al, 1994). With this material, it is possible to study the PC mycelium. As data about PC chemical composition are not available in the literature, the first aim of this paper was to produce large amounts of standard film and to characterize chemically PC mycelium.

Actual PC mycelium from cheese exists in two states. In the cheese factory, the raw mycelium is thick and free. After cheese conditioning, the mycelium is compressed between the cheese crust and the packaging film; its structure is then thin and compact. Our second objective was to study the mechanical and transfer properties of raw and compressed mycelium film.

MATERIALS AND METHODS

Medium to simulate the soft cheese

The composition of the medium is presented in table I. The medium was adjusted at pH 4.8, sterilized, degassed and spread in a rectangular tray (40 cm × 25 cm × 1 cm).

Film production

4 10^9 freeze-dried spores of *P camemberti* A (Texel, Paris, France) were suspended in 500 mL of distilled water containing 4.75 g of trypton salt broth (Biokar SA, Beauvais, France) and 0.5 g of anhydrous glucose (Merck, Darmstadt, Germany). After 16 h of hydration at 10°C, 100 mL of spores suspension were spread on the medium to get 8 10^5 viable spores/cm^2.

Incubation lasted for 6 days in a regulated chamber at optimum conditions, ie, 25°C and 96% relative humidity (RH). The film of PC mycelium, called 'raw mycelium', was easily separated from the medium. The physical hand-

Table I. Medium composition.
Composition du milieu de culture.

Component	*Quantity for 100 mL*
Bacteriological agar (Biokar)	2.17 g
Lactose powder (90%) (Lacto-Serum France)	9.65 g
Sodium chloride (Prolabo)	2.17 g
Ammonium sulfate (Prolabo)	0.11 g
Sodium lactate (Prolabo)	0.54 g
Lactic acid N/100 (Prolabo)	1.74 mL

lings were done carefully to limit damage to the PC sheet. A part of the PC mycelium was laminated with a 10-kg smooth stainless steel roll (pressure 2.5 10^{-5} Pa) to get a laminated film similar to the *Penicillium* film compressed between the cheese and its packaging.

Physicochemical analysis

Before analysis, PC mycelium was conditioned at 20°C and 68% RH for 24 h according to ISO 187 standard for paper and board.

The water content was determined by drying 5 h at 105°C (ISO 287). The total lipid amount was measured using the methanol-chloroform-water method (gravimetric method described by Lecoq (1965)). The total and non-protein nitrogen was dosed by the Kjeldhal method after mineralizing.

To measure mineral content, 1 g of the mycelium was placed 12 h at 520°C and the ashes were weighed. To dose the copper and iron content, the ashes were dissolved in 1 mL of nitric acid and adjusted to 5 mL with distilled water. To dose the calcium, sodium, magnesium and potassium, the ashes were dissolved in 1 mL of hydrochloric acid, 0.5 mL of lanthan chloride and adjusted to 5 mL with distilled water. All minerals were dosed with an atomic absorption spectrophotometer 1100 (Perkin Elmer, Montigny-le-Bretonneux, France).

The carbohydrate content was measured using the chromometric method described by Dubois et al (1956) with a spectrophotometer Ultrospec III (Pharmacia, London, UK). The sulfuric acid/phenol solution produces a yellow coloring with carbohydrates. After extraction in sulfuric acid, the cellulose content was determined by the classical chromometric method described by Updegraff (1969).

All results were the mean of triplicate measurements. When standard deviation (SD) is not mentioned, its value was less than 5% of the mean.

Physical properties

Thickness was measured using a micrometer 1601MX (Messmer, London, UK) according to the ISO 534. Mycelium density was determined by weighing (ISO 536). The maximum tensile strength and elongation measurements were done with an universal testing machine 1122 (Instron, London, UK) according to ISO 1924. The water vapor permeability (WVP) of the mycelium film was measured with a L 80-4000E (Lyssy, Zollikon, Switzerland) according to ISO 2528. Gas permeability was measured with a L 100-2402 (Lyssy, Zollikon, Switzerland) according to ASTM D1434. The method used for water sorption isotherm (WSI) determination was based on equilibration with saturated salt solutions (Desobry and Hardy, 1993).

RESULTS AND DISCUSSION

Production of raw PC mycelium

PC mycelium had a surface density of 152 ± 12 g/m^2, with 115 ± 5 g of dry matter. The raw mycelium was dense, homogeneous and had an average thickness of 2.0 ± 0.2 mm. The laminated PC film was 0.27 ± 0.03 mm. The external side of the film was white and the slightly yellowish color of the side facing the agar plate was probably due to the medium coloring. The films obtained were similar in thickness and surface density to the actual mycelium on soft cheeses before and after packaging. The production process simulated well the PC growing on cheese.

Chemical composition of the PC mycelium (table II)

The water content of the laminated mycelium was 25%. The water content could vary because of treatment or osmotic pressure (Kockova-Kratochvilova, 1990). Because of the standard condition of growth, in our experiments, water content was stable.

The total protein content was included in the same range as 11 different fungi strains reported by Cavazzoni and Adami (1992) or two different *Penicillium* species studied by Cochrane (1958). However, *Penicillium commune*, a wild ancestor of *P*

Table II. Total composition of *P camemberti* mycelium.

Composition globale du mycélium de P camemberti.

Component	*Amount (%)*
Water content	25 ± 2
Proteins	8.6 ± 1.1
Lipids	25.2 ± 2.2
Glucides	33.3 ± 2.1
including cellulose	5.5
Ashes	4.5 ± 0.3
Undetermined	3.4

Table III. Mineral composition of *P camemberti* mycelium.

Composition minérale du mycélium de P camemberti.

Mineral	*mg/100 g of dry matter*
Magnesium	30 ± 4
Potassium	390 ± 27
Calcium	7 ± 1
Natrium	910 ± 127
Phosphorus	1.20 ± 0.06
Iron	2.0 ± 0.7
Copper	0.35 ± 0.11

camemberti, contains up to 40% of proteins (Garcia, 1982) but this amount might be overestimated because of the high non-protein nitrogen sources such as chitin or nucleic acids. Nevertheless, Solomons (1975) used *P chrysogenum* to produce proteins for human consumption because of its protein content (15% dry basis). This amount is similar for *P camemberti*. Solomons (1975) calculated protein amount up to 47% for fungi. More recently, Barker et al (1982, 1983) used filamentous fungi to produce proteins and obtained up to 52 $g_{crude\ protein}$/100 g.

The lipid content was three times higher than the protein content, in agreement with Fanni (1977) who reported an amount of 23.5% in the same mould growing on cheese. Cochrane (1958) showed a high variability of lipid content from 1.4% to 34.5% dry basis for *P chrysogenum* and *P griseofulvum*, respectively. Some authors reported a lipid content up to 60% dry basis. Compared with yeast, the major difference appears in the lipid content which is five times higher for *P camemberti* than baker's or fodder yeasts (Kockova-Kratochvilova, 1990).

Contents of cellulose and total carbohydrates agree with the results of Garcia (1982) with 10% of cellulose in *P commune* and Cavazzoni and Adami (1992) estimating the carbohydrate content between 30 and 61.2%. Concerning ashes, *P camemberti* was positioned with *Agaricus* and *Candida* (Hayes, 1978). The minerals represented 3.4% of dry weight (table III). Amounts of potassium and sodium agree with the results of Hayes (1978). Some other species of *Penicillium* present a mineral composition strongly different from *P camemberti* (Cochrane, 1958).

Mechanical properties

The maximum tensile strength of PC mycelium was 0.266 kN/m (SD = 7%), 10 times less than a standard paper or plastic of the same thickness. The film elongation calculated as the maximum length of the film before it breaks divided by the initial length was 1.33 versus 1.02 for the packaging papers and could be explained by the low interaction between mycelium fibers compared to cellulose fibers. Industrially, the high elasticity allows a high resistance to cheese deformation during the process. When conditioned, the soft cheeses are compressed and their diameter is reduced to 90%

of the initial diameter to fit in the wood or paperboard box. The high elasticity of the PC film allows this process without any structural alteration of the PC film.

Water sorption isotherms

Figure 1 shows that PC water content increases exponentially for a_w above 0.75. At 20°C, the maximum water absorption of the mycelium is 175% of dry mycelium weight, which corresponds to 237 g_{H2O}/m^2_{film}. During processing and cheese sale, temperature varies from 4°C to 25°C (or higher during summer). Cheese storage into unrefrigerated areas leads to vaporizing of water that condenses if temperature decreases. PC mycelium fixes the condensed water until the packaging is able to absorb it. Nevertheless, if high water amounts are retained for a long period, physiological alterations occur to PC.

Moreover, during isothermal storage, water content in the PC is largely higher on cheese surface (a_w = 0.97) than on packaging surface (a_w = 0.90). As shown in figure 1, small variations of water activity (a_w) lead to large changes of water content for a_w range between 0.90 and 0.97. This can modify the breathing of the mould but the effect of water content on PC metabolism was not the objective of the present paper.

Water and gas transfer properties

The water vapor permeability (WVP) of the raw mycelium was six times higher than the WVP of the laminated mycelium (table IV). As compared to other edible packaging materials, the laminated mycelium WVP was equivalent to a maltodextrin film (4.7 10^{-11} kg/m/s/Pa), 2.5 times lower than a pectin film, 1.5 times lower than a starch

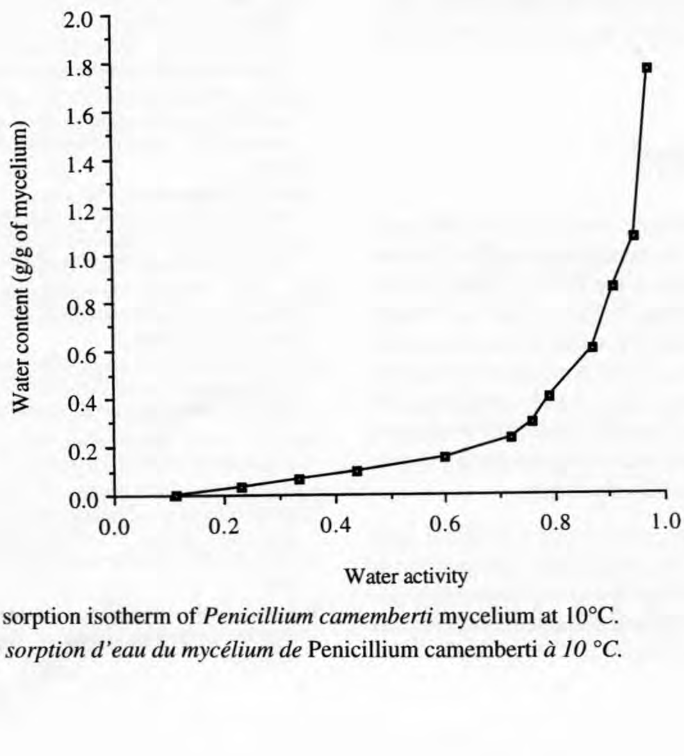

Fig 1. Water sorption isotherm of *Penicillium camemberti* mycelium at 10°C.
Isotherme de sorption d'eau du mycélium de Penicillium camemberti *à 10 °C.*

Table IV. Water vapor transmission rate (WVTR) and water vapor permeability (WVP) of mycelium of *P camemberti*.

Vitesse de transfert d'humidité et perméabilité à la vapeur d'eau du mycélium de P camemberti.

Mycelium	*WVTR* *(kg/m²/s)* *38°C – 90% ΔRH*	*WVP* *(kg/m/s/Pa)* *38°C*
Raw	$2.6\ 10^{-5}$	$24.2\ 10^{-11}$
Laminated	$3.4\ 10^{-5}$	$4.2\ 10^{-11}$

film but largely higher than lipid films (Guilbert and Biquet, 1986).

Concerning oxygen or CO_2 permeability through raw and laminated PC mycelium, no measurement was technically possible because of an extremely high permeability. We can assume that no limiting effect on gas transfer occurs in PC mycelium. During the storage, surface mould has no significant influence on gas exchanges between the cheese pasta and the packaging. Breathing is not limited by gas transport through mycelium.

CONCLUSION

The *P camemberti* mycelium can be considered as the first wrapping material around the Camembert or the Brie. Its WVP is high but comparable to those of other edible films, like maltodextrin, pectin or starch. No limitation of gas transport was observed. The structure of the laminated PC mycelium is visually similar to packaging paper. PC has interesting mechanical properties, especially elasticity.

The surface mould plays multiple roles and further investigations are needed to understand completely the preservation and the ripening of these soft cheeses during storage.

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