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Bioformation of flavour by *Penicillium candidum*, *Penicillium nalgiovense* and *Geotrichum candidum* on glucose, peptone, maize oil and meat extract

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Geotricum candidum may be useful as a flavour-improving starter culture in fermented meat products. Therefore, the formation of volatile compounds from *G. candidum* (ATCC 55692) and *G. candidum* (CD 1) on solidified media containing either glucose, peptone, maize oil or meat extract were compared to the commercial starter cultures *Penicillium nalgiovense* (Edelschimmel Kulmbach 72) and *P. candidum* (Super Actif). The *Geotrichum* spp. did not produce significant volatile compounds on meat extract, whereas they produced 3-methylbutanol, 2-methylbutanol, 3-methylbutanal, 2-methylpropanol and dimethyldisulfide on glucose, peptone and maize oil. The *Penicillium* spp. produced 1-octen-3-ol, diacetyl/butan-2-one, pentan-2,3-dione, methyl branched alkene, octan-3-one and decanal on all substrates. It could be concluded that the use of *Geotrichum* spp. as flavour-improving starter cultures rely on growth of the culture and that the produced volatile compounds are independent of the composition of the substrate. © 1997 Elsevier Science Ltd

INTRODUCTION

Mould starter cultures play a very important role in the formation of flavour in foods fermented with fungi (Kinderlerer, 1989). In cheese products it is often a variety of different species of fungi such as *Penicillium* spp. (Galloway & Crawford, 1985) and *Geotrichum candidum* (Jollivet *et al.*, 1994) which is used, whereas mainly *Penicillium nalgiovense* is used in meat products fermented with fungi (Lücke, 1994). To improve the flavour of fermented meat products it is therefore obvious to use a wider range of different fungi. Jollivet *et al.* (1994) have shown that strains of *Geotrichum candidum* are able to produce volatile compounds, which are highly relevant for the flavour of high quality dry-cured hams (Hinrichsen & Pedersen, 1995). Consequently, *Geotrichum* spp. may be useful to improve the flavour of fermented meat products.

Dairy and meat products are very different as they consist of complicated matrices formed by protein, lipid and carbohydrates. All these components may contribute to the flavour formation by serving as substrates for the micro-organisms. In this way, the composition of the food may influence the flavour formed by the fermenting micro-organisms as the specific substrates lead to specific flavour components.

The impact of the mould strains has largely been explained by general hydrolytic activities by lipases and proteinases (Trigueros *et al.*, 1995). These activities lead to the liberation of non-volatile compounds such as free fatty acids, low molecular weight peptides and free amino acids, without any impact on the aroma. The stimulation of the epithelial receptor cells in the human nose are only affected by molecules released to the gaseous phase in the oral cavity by mastication and compounds with aroma characteristics therefore have to be volatile. Hydrolytic reactions by themselves will in this way have very little impact on the aroma. But recent studies have demonstrated that moulds give rise to a wide range of volatile compounds as a result of secondary metabolism (Jollivet *et al.*, 1994; Pastore *et al.*, 1994; Larsen & Frisvad, 1995; Sunesson *et al.*, 1995) and such activities reveal the flavour improving potential of moulds in mould fermented foods.

The aim of the present study was to investigate the possibilities of using two different strains of *Geotrichum*

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candidum as starter cultures for mould fermented meat products by evaluating the formation of volatile compounds. The *Geotrichum* strains were compared to two commercial mould starter cultures. Additionally, the impact of substrate composition was investigated on four different media containing either glucose, peptone, maize oil or meat extract, respectively.

MATERIAL AND METHODS

Cultures

The fungi used were *Geotrichum candidum* (ATCC 55692), *Geotrichum candidum* (CD 1) from Sochal (France), *Penicillium candidum* (Super Actif) from Chr. Hansen A/S (Denmark) and *Penicillium nalgiovense* (Edelschimmel Kulmbach 72) from Rudolf Müller & Co. GmbH (Germany).

The *G. candidum* strains were maintained as described previously (Kier *et al.*, 1976).

The Penicilliums were obtained as lyophilized cultures, and were used directly as delivered.

The fungi were cultivated in 9-cm Petri dishes with 25 ml of the different medias at 20°C for 4 to 7 days.

Growth media

The growth media were made with a basic salt medium and three different carbon sources. The basic salt medium was a modification of the DM medium described by Trinci (1971) and consisted of (g/l): KH_2PO_4 3.40, $Na_2HPO_4.7\ H_2O$ 6.66, $(NH_4)_2SO_4$ 6.00, NaCl 30.00. pH was adjusted to 5.8 before autoclaving at 121°C for 20 min. EDTA-chelated trace elements consisted of (g/l): EDTA 0.6, $MgSO_4.7\ H_2O$ 0.25, $CaCl_2$ 0.05, $ZnSO_4.7H_2O$ 0.2, $MnSO_4.4\ H_2O$ 0.02, $CuSO_4.5\ H_2O$ 0.005, $FeSO_4.7\ H_2O$ 0.1, Na_2SO_4 0.5, $NaMoO_4.2H_2O$ 0.005. The carbon sources were per. l: Glucose 10 *g*, Bacto Peptone 30 *g* or Maize oil 6 *g* (local supplier) emulsified with 1.2 *g* gum arabic. The maize oil was emulsified in a Nelco Omnimixer for 5 min at maximal speed by mixing a solution of 0.6 *g* gum arabic in 90 ml of water with 3 *g* maize oil which afterwards was diluted to the appropriate concentration. The EDTA-chelated trace elements were prepared separately at 20 times their final concentration, and the salt medium and the carbon sources were prepared separately in double concentration.

The media were solidified by adding agar (Oxoid Purified Agar code L 28) to a final concentration of 1.5%. The agar was autoclaved together with the carbon sources. Meat extract was prepared by mixing 30 *g* of pork (*m. longissimus dorsi*) cut into 1×1×1 cm pieces with 60 ml of 0.03 M KH_2PO_4 adjusted to pH 5,8 and 3% NaCl. The mixture was homogenised in a Nelco Omnimixer for 1 min. The process was repeated until the desired amount of meat extract was obtained. The meat extract was centrifuged at 2100 *g* for 20 min at 5°C. The supernatant was collected and mixed with an equal amount of potassiumphosphate buffer. This mixture were centrifuged at 46000 *g* for 60 min at 5°C. The supernatant was filtered through a 0.22 μm sterile membrane. The sterile meat extract was heated to 45°C and 3 vol. of meat extract mixed with 1 vol. of a 6% agar solution.

pH and NaCl concentration in the media with glucose, peptone, maize oil and meat extract were selected to simulate conditions found in fermented meat products.

The plates were inoculated by spreading 0.1 ml of a conidia suspension ($OD_{620}=0.5$) on each plate. The conidia from the *G. candidum* strains were harvested from the agar plates with a swab stick and suspended in sterile water with 0.9% (w/v) NaCl. Four plates were made for each strain and substrate.

Gas chromatographic analyses

The extraction of volatile compounds, head space gas chromatography flame ionization detection (HSGC-FID) and head space gas chromatography mass spectrometry (HSGC-MS) were performed as described by Hinrichsen and Pedersen (1995) except that the entire agar medium with the different fungi were cut into 1×1 cm pieces and the content of one Petri dish transferred to the 500-ml conical flask. The samples for HSGC-FID were made in triplicate and 1 of each sample was used for HSGC-MS.

Statistical methods

Results from gas chromatography were examined by principal component analysis (PCA) and subsequently soft independent modelling of class analogy (SIMCA) in the Unscrambler (CAMO A/S, 1994). The experiment was repeated twice.

RESULTS AND DISCUSSION

Growth of the fungi was evaluated visually on the Petri dishes and when an entire Petri dish was covered with mycelium the cultures were harvested. All the fungi had the fastest growth on the peptone medium and were harvested after four days. On glucose, the fungi were harvested after five days. Growth on the maize oil containing medium was less pronounced than on glucose and peptone media, and the mycelium was less dense. The fungi were harvested after six days on this medium. On the meat extract medium, the two strains of *G. candidum* grew very slowly and even after seven days of incubation the mycelium did not cover the entire surface of the agar Petri dish. The penicilliums grew somewhat better and were more dense than the *G. candidum* strains on meat-extract medium. All the cultures on meat extract were harvested after seven days regardless of their growth.

In Table 1, results of GC-MS are shown. In total, 56 volatile compounds were detected of which 53 were identified. Among these there were 13 aldehydes, 12 alcohols, 1 ester compound, 8 ketones, 2 carboxylic acids, 2 hetero cyclic compounds, 2 nitrogen compounds, 4 sulphur compounds, 8 hydrocarbons and 3 unidentified compounds. This is partly in accordance with the studies of Pastore *et al.* (1994) and Jollivet *et al.* (1994) of *Geotricum* spp. grown on complex media and milk and cheese media. A larger representation of ester compounds was, however, observed in these studies. Different *Penicillium* spp. were investigated on DG18 medium or malt-extract agar by Sunesson *et al.* (1995). On malt-extract agar many ester compounds were observed, whereas terpenoids were observed on DG18 medium. None of these compounds could be detected in the present investigation for either *Geotrichum* spp. or *Penicillium* spp. This may be due to a short incubation period in the present study, the strains involved, differences in the environmental conditions and/or the analytical techniques. Larsen and Frisvad (1995) demonstrated that the formation of terpenoids takes place after longer incubation periods in *Penicillium* spp, and Sunesson *et al.* (1995) incubated the investigated strains for two weeks. Furthermore, results of Sunesson *et al.* (1995) indicate the importance of water activity for *Penicillium commune*. Grown on a substrate with low water activity, *P. commune* forms terpenoids, whereas on malt extract agar a high number of ester compounds are produced. Additionally, terpenoids are less volatile and, as the applied isolation procedure of volatile compounds is based on more volatile compounds, the less volatile terpenoids may not be detected. In the study of Larsen and Frisvad (1995), diffusive sampling was used for isolation of volatile compounds. This technique is cumulative and therefore very sensitive.

The representation of many different chemical groups in Table 1 indicates a diverse metabolism of the examined fungi. However, only a limited number of compounds varied according to the type of strain and substrate, as demonstrated in Figs 1(a) and (b).

Peak areas of the volatile compounds were exposed to principal component analysis in order to deconvolute the very complex gas chromatographic data. In Figs 1(a) and (b) the quantitative part of the gas chromatographic analyses are presented after principal component analysis as scores and loadings plot, respectively. The resulting model describes 29% of the variation by the first principal component and 18% by the second, thus 47% of the total variation in the data set was described. Data were normalized (divided by the mean peak area for each chromatogram). In this way, differences in the quantities of volatile compounds were eliminated and effects as a result of different growth rates of the fungi on the substrates could be excluded during the interpretation of data. Furthermore, the square root of the data was used in order to bring data within the same range. Ethanol (KI 560) and an amine (KI 585) were omitted in the analysis because these two peaks dominated the gas chromatograms and were not entirely separated. These two volatile compounds were not characteristic for any of the fungi on the different substrates (results not shown).

The scores plot in Fig. 1(a) reveals three groupings designated I to III. The corresponding loadings plot is shown in Fig. 1(b) and it appears that the volatile compounds are mainly displaced in three directions.

The observed groupings in Fig. 1(a) were tested statistically by soft independent modelling of class analogy (SIMCA) and all three groupings differed significantly ($P < 0.05$). In Table 1, volatile compounds important for each group (modelling power > 2.5) are indicated by the number of the characteristic group. Further, volatile compounds with loadings $> |0.1|$ are marked. These compounds are decisive for the differences among the three observed groupings by principal component analysis.

Group I in the scores plot in Fig. 1(a) is composed of control samples for meat extract, glucose, peptone and maize oil situated together with *Geotrichum candidum* ATCC 55692 and *Geotrichum candidum* (CD 1) grown on meat extract. This indicates that these two strains do not produce any specific volatile compounds with meat extract, as the substrate compared to uninoculated samples. However, as these two strains had poor growth on meat extract, it may be that this substrate is inadequate or inhibitory for the investigated strains and therefore does not support the metabolism of the *Geotrichum* spp. These findings are of particular relevance for meat fermentations as these two strains seem to be unsuitable as flavour improving cultures in high-protein products.

In group II, the same two strains of *Geotrichum* spp. are placed but with peptone, glucose or maize oil as substrate. Disregarding the composition of the substrate these two strains produce the same pattern of volatile compounds when grown on either peptone, glucose or maize oil (Fig. 1(a)). The loadings plot in Fig. 1(b) reveals that the volatile compounds produced is 3-methylbutanol (KI 853), 2-methylbutanol (KI 854), 3-methylbutanal (KI 736), 2-methylpropanol (KI 740) and dimethyldisulfide (KI 808). All these compounds may be products of amino acid catabolism (Hemme *et al.*, 1982). However, on the media with glucose and maize oil, the content of free amino acids and proteins is very low or even absent. On these substrates, the volatile compounds, therefore must be a result of *de novo synthesis* with either glucose or triglyceride as carbon source. The metabolic activities of strains placed in group II seems, therefore, mainly to be catabolism of free amino acids on peptone and synthesis *de novo* for glucose and maize oil. In addition, no volatile compounds derived from glucose or fatty acids could be observed for these two strains on the substrates with glucose and maize oil. It is well known that *G. candidum* produces lipases on synthetic media with triglycerides as the only carbon source (Jacobsen *et al.*, 1989). In this

Table 1. Identification of volatile compounds from substrates containing glucose, peptone, maize oil or meat extract inoculated with either *Geotrichum candidum* (CD1), *Penicillium nalgiovense* (Edelschimmel Kulmbach 72), *G. candidum* (ATCC 55692) or *P. candidum* (Super Actif)

	Compound	Kovats' index	Reliability[a]	Loadings > |0.1|	Group[b]
Aldehydes	Acetaldehyde	2.16	c		ns[c]
	Propanal	572	b	*	II
	Butanal	629	b		II
	2-Methylpropanal	638	b		ns
	3-Methylbutanal	736	a	*	II III
	Pentanal	780	b	*	ns
	Hexanal	886	b		III
	Heptanal	989	b	*	ns
	2,4-Nonadienal	1031	c		ns
	Benzaldehyde	1085	a		ns
	Octanal	1090	a	*	II III
	Nonanal	1198	b	*	ns
	Decanal	1290	b	*	ns
Alcohols	Ethanol	560	a	ni[d]	ns
	Isopropanol	620	a	*	II
	n-Propanol	668	a		ns
	2-Butanol	710	a		ns
	2-Methylpropanol[f]	740	a	*	II
	3-Penten-1-ol	847	c		II
	3-Methylbutanol	853	a	*	II
	2-Methylbutanol	854	a	*	II
	Pentanol	882	b		ns
	3-Penten-2-ol	891	c		ns
	1-Octen-3-ol	1082	a	*	III
	3-Decyne-2-ol	50.42	c		II
Ester compounds	3-Octen-1-ol-acetate	51.64	c		ns
Ketones	Acetone	603	a	*	ns
	Diacetyl/butan-2-one	684	a	*	ns
	Pentan-2-one	777	b		ns
	Pentan-2,3-dione	792	c		III
	Heptan-2-one	982	b		ns
	4-Octen-3-one	1064	c		ns
	Octan-3-one	1072	c		ns
	Nonan-2-one	1187	b		ns
Carboxylic acids	Acetic acid	815	a		ns
	3-Methylbutanoic acid	1018	a		ns
Hetero compounds	2-Methylfuran	652	c		III
	1-Methylene-1H-indene	47.04	c	*	ns
Nitrogen compounds	Amine	585	d	ni	ns
	O-decylhydroxylamine	52.39	c	*	ns
Sulfur compounds	Ethanethioic acid-S-methylester	767	c		II
	Dimethyldisulfide	808	a	*	II
	Dimethyltrisulfide	1052	b		II
	Methyl-N-penthyldisulfide	1208	c		ns
Hydrocarbons	Benzene	711	c		ns
	Methyl branched alkene	754	d	*	ns
	1-Octene	801	c		II
	Toluene	817	a		ns
	Nonene	901	c		II III
	Dimethylbenzene	918	c		ns
	Dimethylbenzene	921	c		ns
	Methyl branched alkene	52.58	d		ns
Unknowns	Unknown 1 (MP[e] = 104, 78, 51)	960	d		ns
	Coelution	1094	d	*	II III
	Unknown 2 (MP = 43, 95, 67)	51.46	d		III

[a]Reliability of identification is indicated by: a, mass spectrum and Kovats' index identical to authentic compound; b, mass spectrum and Kovats' index in agreement with corresponding literature data; c, mass spectrum consistent with spectrum found in the NIST library 1990 version Finigan MAT; d, tentative identification by mass spectrum or unidentified.
[b]Significant differences ($P < 0.05$) between groupings (I-III) found by principal component analysis and tested by soft independent modelling of class analogy.
[c]ns, not significant.
[d]ni, not included in the statistical analysis.
[e]Major peaks in mass spectrum in decreasing order.
[f]For uninoculated peptone samples this peak was identified as 2-methylbutanal (b).

context, the lack of degradation products from free fatty acids in the present study is remarkable. In model cheese medium, Jollivet *et al.* (1994), detected such volatile compounds though only in trace amounts and highly dependent on strain.

For *Geotrichum candidum* ATCC 55692 and *Geotrichum candidum* (CD 1) on glucose, maize oil or peptone it seems that the substrate composition is unimportant under the present conditions. The two strains grew well on the peptone substrate and this indicates that the reason for the lack of growth on meat extract could be the size of the proteins. On the meat extract the native proteins are large (Ouali, 1992), whereas the proteins are degraded to smaller peptides and free amino acids on peptone. If the strains do not posses sufficient extra cellular proteolytic activity, they will not be able to

Fig. 1. Score plot (a) and loading plot after principal component analysis of volatile compounds. In (a) first letter designates substrate: F, maize oil; G, glucose; K, meat extract and P, peptone. Next two letters designate strain: CD, *Geotrichum candidum* (CD1); EK, *Penicillium nalgiovense* (Edelschimmel Kulmbach 72); GC, *G. candidum* (ATCC 55692) and PC, *P. candidum* (Super Actif). Last number designates replicates. In (b) numbers assign Kovats' indices.

Table 2. **Quantities (ng dodecane equivalents) of discriminating volatile compounds. First letter designates substrate: F, maize oil; G, glucose; K, meat extract and P, peptone. Next two letters designate strain: CD, *Geotrichum candidum* (CD1); EK, *Penicillium nalgiovense* (Edelschimmel Kulmbach 72); GC, *G. candidum* (ATCC 55692) and PC, *P. candidum* (Super Actif). $n = 3$**

Compound	Kovats' index	F	FCD	FGC	FEK	FPC	G	GCD	GGC	GEK	GPC	K	KCD	KGC	KEK	KPC	P	PCD	PGC	PEK	PPC
Diacetyl/butan-2-one	684	13.7	5.1	3.4	24.9	74.4	16.1	10.1	3.7	45.8	58.3	8.8	5.2	1.1	31.9	6.5	161.8	36.2	24.1	367.9	160.7
3-Methyl-butanal	736	0.0	28.6	34.2	7.3	66.1	0.0	25.1	53.9	17.6	71.4	3.6	12.2	15.0	0.0	0.0	341.8	23.6	27.4	6.5	46.7
2-Methyl-propanol	740	6.8	66.3	110.5	8.1	16.3	6.3	74.7	142.2	11.7	43.2	12.6	9.4	15.4	11.5	11.9	84.4[a]	31.8	10.2	10.4	24.9
3-Methyl-butanol	853	0.0	168.2	256.2	15.6	9.9	0.9	142.5	349.0	6.9	3.8	1.0	7.7	19.7	4.2	3.3	0.0	106.6	84.3	15.2	97.6
2-Methyl-butanol	854	0.0	84.0	128.1	11.7	5.7	0.0	82.3	177.2	3.8	5.6	0.0	2.6	9.5	4.5	2.0	0.0	33.9	9.7	10.8	1.1
Octan-3-one	1072	0.0	0.0	0.0	3.5	3.9	0.0	0.0	0.0	8.4	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	6.7
1-Octen-3-ol	1082	6.5	11.0	3.7	322.9	254.7	2.1	1.8	1.1	188.8	157.9	1.1	4.7	3.8	218.2	174.2	1.4	0.0	0.0	750.1	313.0
Decanal	1290	0.9	0.0	0.0	99.2	1.3	0.0	0.0	1.1	24.3	0.0	0.0	3.8	2.7	33.1	3.1	0.0	2.9	1.8	64.5	0.0

[a]For uninoculated peptone samples this peak was identified as 2-methylbutanal.

utilize the proteins in the meat extract. In *Geotricum candidum* strain 96C, Hannan and Gueguen (1985) revealed activity of a protease with endopeptidasic activity on casein. Meat proteins are, though, very different from casein (Regenstein & Regenstein, 1984) and the detected protease may not be able to degrade meat proteins or the employed strains may not possess proteolytic activity.

The similarity of volatile compounds produced by *Geotrichum* spp. on peptone, glucose and maize oil is important for meat fermentation. It is apparently not possible to manipulate the flavour produced by these strains in a fermented meat product on the basis of product composition. As these strains do not grow on high-protein products, it is likely that they will only operate in a partly decomposed meat product with either maize oil and/or glucose added and the volatile compounds produced will be end products of amino acid catabolism.

All the samples with *Penicillium candidum* and *Penicillium nalgiovense* grown with glucose, peptone, maize oil or meat extract are placed in group III in the scores plot in Fig. 1a. The grouping of these two strains on all tested substrates shows that the importance of the composition of the substrate is negligible regarding the formation of volatile compounds. The loadings plot in Fig. 1b reveals that the volatile compounds characteristic for group III are 1-octen-3-ol (KI 1082), diacetyl/butan-2-one (KI 684), pentan-2,3-dione (KI 792), methyl branched alkene (KI 754), octan-3-one (1072) and decanal (KI 1290). Diacetyl (KI 684) is formed from glucose by conversion of pyruvate (Romano & Suzzi, 1996), whereas the remaining volatile compounds are all secondary degradation products from triglycerides (Kinderlerer, 1989). Only in the glucose medium is there glucose present. Therefore diacetyl is synthesized *de novo* on meat extract, peptone and maize oil. Likewise there are only triglycerides present in the medium with maize oil and therefore 1-octen-3-ol (KI 1082), pentan-2,3-dione (KI 792), methyl branched alkene (KI 754), octan-3-one (KI 1072) and decanal (KI 1290) are formed *de novo* on glucose, peptone and meat extract.

For the use of *Penicillium candidum* and *Penicillium nalgiovense* in meat fermentation, the present results indicate that the composition of the product, is of no importance and that it is possible to use these strains as starter cultures in many different types of products. Regardless of the composition of the product, these strains will form degradation products of free fatty acids.

In Table 2, the absolute amounts of the referred volatile compounds are listed. For *Geotrichum candidum* ATCC 55692 and *Geotrichum candidum* (CD 1) the amount of 3-methylbutanal (KI 736) ranges from 12.2 to 53.9 ng dodecane equivalents, 2-methylpropanol (KI 740) from 9.4 to 142.2 ng dodecane equivalents, 3-methylbutanol (KI 853) from 7.7 to 349.0 ng dodecane equivalents and 2-methylbutanol (KI 854) from 2.6 to 177.2 ng dodecane equivalents, whereas the amount of 1-octen-3-ol ranges from 0.0 to 11.0 ng dodecane equivalents. For *Penicillium candidum* (Super Actif) and *Penicillium nalgiovense* (Edelschimmel Kulmbach 72), the amount of 3-methylbutanal (KI 736) ranges from 0.0 to 71.4 ng dodecane equivalents, 2-methylpropanol (KI 740) from 8.1 to 43.2 ng dodecane equivalents, 3-methylbutanol (KI 853) from 3.3 to 97.6 ng dodecane equivalents and 2-methylbutanol (KI 854) from 1.1 to 11.7 ng dodecane equivalents, whereas the amount of 1-octen-3-ol ranges from 157.9 to 750.1 ng dodecane equivalents.

The amount of 3-methylbutanal (KI 736) and 2-methylpropanol (KI 740) in the control samples of peptone is very large compared to those of the inoculated peptone samples (Table 2). Mass spectrometric analyses showed that the compound with KI 740 for the uninoculated peptone samples was not 2-methylpropanol but 2-methylbutanal which, in the inoculated samples, was not possible to determine. The large amount of 3-methylbutanal (KI 736) and 2-methylbutanal (KI 740) in the control samples may be explained by the heat treatment (121°C for 20 min), as these two aldehydes can be formed by Strecker degradation of free amino acids in the presence of molecules with vicinal diketones (Belitz & Grosch, 1986). It seems, though, that the fungi degrade or adsorb these two aldehydes as the amount in the inoculated peptone samples is lower than in the uninoculated samples.

CONCLUSION

The present results indicate that the exclusive factor of importance for formation of volatile compounds is the species and not the substrate, provided that it supports growth. For *Geotrichum candidum* ATCC 55692 and *Geotrichum candidum* (CD 1), volatile compounds derived from amino acid catabolism are formed whereas, for *Penicillium candidum* (Super Actif) and *Penicillium nalgiovense* (Edelschimmel Kulmbach 72), volatile compounds derived from free fatty acids are formed. As the *Geotrichum* spp. had weak growth on the meat extract media compared to *Penicillium candidum* (Super Actif) and *Penicillium nalgiovense* (Edelschimmel Kulmbach 72), it is likely that the use of *Geotrichum* spp. as flavour-improving starter cultures rely on the composition of the substrate, as degraded proteins, fatty acids or glucose is necessary for flavour production from these strains.

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