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Identification of Volatile Metabolites from Rice Fermented by the Fungus *Monascus purpureus* (Ang-kak)

P. PATÁKOVÁ-JŮZLOVÁ[a]*, T. ŘEZANKA[b] and I. VÍDEN[a]

[a]*Institute of Chemical Technology, 166 28 Prague 6, Czech Republic*
[b]*Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Czech Republic*
Fax +420 2 2431 1082
E-mail petra.juzlova@vscht.cz

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ABSTRACT. Eighty volatile compounds that create typical ang-kak aroma have been identified in samples of rice grains fermented by *Monascus purpureus* and in cultivation media after *M. purpureus* fermentation. These volatile metabolites include alcohols, aldehydes, ketones, esters and terpenoid compounds. Identification has been performed by means of GC–MS after sample distillation and extraction with dichloromethane.

The fungus *Monascus* from the class *Ascomycetes* is commonly used for the production of red rice known as ang-kak in Asian countries (Jůzlová *et al.* 1996*a*). Ang-kak, rice fermented with *Monascus* (in a form of dried powder), is traditionally utilized for the coloring of food, especially fish, cheeses and alcohol beverages in Asia but it is also becoming increasingly popular with European meat product producers as a coloring agent for salami and sausages (Fabre *et al.* 1993). Ang-kak is usually produced according to empirically found rules and so far, this fermentation has never been described completely using scientific parameters. One of the unknown parameters of ang-kak production is a development of a specific aroma that can serve as an indicator of well-done cultivation (Hesseltine 1965). This aroma, varying in the course of the fermentation, can resemble apple odor (Jůzlová 1994).

Identification of ang-kak volatiles can help to map *Monascus* metabolism, can facilitate recognition of success or poor success of ang-kak fermentation, and, last but not least, can be interesting for meat product producers because the volatile compounds can affect the odor of final meat products. This article also points out differences between the formation of the volatile compounds on rice grains and in a liquid medium.

MATERIALS AND METHODS

Cultivation. Monascus purpureus strain CCM 8152, maintained on malt extract agar, was cultivated either in 300-mL Erlenmeyer flasks containing 50 mL of culture medium on a reciprocal shaker (Jůzlová *et al.* 1994) or in plastic bags containing *ca.* 60 g of moist rice grains (Lotong and Suwanarit 1990). In both cases the cultivation proceeded for 5 d at 30 °C. The culture medium, which was a modified production medium (Lin 1973), consisted of (in g/L) glucose 30, $(NH_4)_2SO_4$ 1.2, $MgSO_4 \cdot 7H_2O$ 1.0, KH_2PO_4 2.5 dissolved in distilled water. The pH after sterilization was *ca.* 5.0. After cultivation, biomass was separated from liquid media by filtration.

Isolation and identification. All samples (in the form of either the filtrate of the cultivation medium or fermented rice grains) were distilled until 20 % of their volume had been collected as distillate. This distillate was then extracted with 10 % (*V*/*V*) of dichloromethane. After drying, the solutions were directly injected into a GC–MS (a double focusing tandem Jeol DX 303, Tokyo, Japan) which was coupled to a 60-m capillary column SPB-5 (ID 0.32 mm). Helium was used as a carrier gas and the oven temperature was held at 50 °C for 2 min and then increased at a gradient of 10 K/min to the final temperature 250 °C. The ionization energy was 11 aJ (70 eV).

*Correspondence author.

RESULTS AND DISCUSSION

The composition of volatile compounds produced by *M. purpureus* on a solid substrate (rice) and in a liquid medium is shown in Table I. Eighty compounds were identified and most of them were present in both types of samples. Ethanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 2,2′-oxydiethanol, 2-methylisoborneol, geosmin, hexadecane, heptadecane and α-bergamotene were the most prominent components in the samples. The determined ethanol content in the samples is probably lower than the actual one because of the method used. 2,2′-Oxydiethanol is probably formed in the samples by the chemical reaction of two ethanol molecules during distillation. Some other compounds (*e.g.* keto-alcohols) may have arisen during distillation in a similar way.

Table I. Volatile compound composition (%) in *M. purpureus* cultivated on solid and liquid media

Compound	Solid	Liquid	Compound	Solid	Liquid
Acetaldehyde	0.13	0.30	Camphene	0.38	0.48
Ethanol	14.80	12.78	Limonene	0.25	0.30
2-Methyl-1-propanal	0.15	0.56	Geosmin	2.14	3.82
1-Propanol	0.83	0.32	2-Methylpentadecane	0.22	0.30
2-Methyl-1-propanol	3.04	4.85	Hexadecane	5.55	6.14
2-Butenal	0.07	0.44	2-Methylbutyrophenone	0.21	0.24
3-Methylbutanal	0.10	0.13	Thujopsene	0.86	1.01
2-Methylbutanal	0.13	0.45	2-Methoxy-3-isopropylpyrazine	0.24	0.30
3-Penten-2-on	0.21	0.48	2-Methylpropyl benzoate	0.30	0.23
3-Methyl-1-butanol	1.96	10.32	Unknown substance	0.32	0.34
2-Methyl-1-butanol	0.96	2.85	Branched heptadecane	0.30	0.34
1-Butanol	0.05	0.52	Pentyl benzoate	0.25	0.34
3-Hexanone	0.80	0.07	β-Caryophyllene	0.18	0.22
3-Methyl-2-pentanone	0.29	0.38	Heptadecane	2.06	2.47
1-Pentanol	0.22	0.14	α-Bergamotene	11.03	13.23
2,4-Pentandione	0.01	0.25	1-Pentylheptyl benzene	0.16	0.19
Hexanal	0.05	0.09	1-Butyloctyl benzene	0.16	0.17
2-Heptanone	0.11	0.33	1-Propylnonyl benzene	0.14	0.17
2-Methylpropyl acetate	0.40	0.21	A branched alkylbenzene[a]	0.39	0.46
2,3,5-Trimethyl-3-hexene	0.25	0.09	Benzyl benzoate	0.00	0.57
2-Heptanol	0.61	0.11	δ-Cadinene	0.25	0.45
2,2′-Oxydiethanol	20.49	6.05	1-Methylundecylbenzene	0.25	0.14
4-Hydroxy-4-methyl-2-pentanone	0.54	0.21	Octadecane	1.02	1.17
1-Heptanol	2.00	0.10	Ethyl myristate	0.09	0.42
3-Methyl-2-heptanone	0.24	0.20	α-Farnesene	0.54	0.12
Benzyl alcohol	0.08	0.93	β-Cubebene	0.15	0.17
2-Ethyl-1-hexanol	0.18	0.44	1-Butylnonylbenzene	0.14	0.14
3-Octanone	0.24	0.22	1-Propyldecylbenzene	0.14	0.17
4-Methyl-3-hexanone	1.51	0.15	An alkylbenzene[b]	0.26	0.34
A multibranched octanol	1.28	0.20	Hexadecane nitrile	0.66	0.76
2-Nonanone	0.38	0.14	1-Methyldodecylbenzene	0.31	0.39
1-Phenylethanol	1.53	0.33	β-Bisabolene	0.62	0.42
2-Nonanol	1.10	0.42	Unknown sesquiterpene[c]	0.78	0.64
2-Nonen-4-on	1.58	1.34	Ethyl palmitate	0.53	1.21
Tetradecane	1.05	0.28	γ-Selinene	0.11	0.09
An alkane	0.40	0.16	Octadecane nitrile	0.48	0.14
2-Methylisoborneol	7.89	9.99	Trichodiene	0.69	0.84
Pentadecane	1.36	1.72	Unknown sesquiterpene	0.13	0.24
5-Ethyl-4-methyl-3-heptanone	0.13	0.64	α-Farnesene	0.77	1.17
cis-Nerolidole	0.15	0.21	β-Selinene	0.24	0.22

[a]M = 246 g/mol. [b]M = 260 g/mol. [c]M = 204 g/mol.

There are interesting differences between the samples from solid state cultivation and submerged liquid cultivation. Thus the content of 3-methyl-1-butanol in the sample from submerged cultivation is five times bigger than that from solid state. However, it can be said that the fungus produces the same metabolites independently of the cultivation type and it is only the amounts of these metabolites that differ depending on the substrate used.

The first group of metabolites (Table I) is formed by alcohols and aldehydes with a low molar mass (*e.g.* ethanol, acetaldehyde, 2-methyl-1-propanol, 2-methyl-1-butanol, 1-phenylethanol), *etc.* These substances are often reported as fungal metabolites (Jolivet and Belin 1993; Sunesson *et al.* 1995) and are also formed in alcohol beverages, for instance in beer, by yeast strains (Engan 1981). They have also been found as the only metabolites in *Monascus* samples prepared by headspace GC (Patáková, *unpublished results*).

3-Octanone and multibranched octanol are compounds that have been identified as metabolites of a number of *Aspergillus* and *Penicillium* species (Karahadian *et al.* 1985). The fungus *Monascus* synthesizes both linoleic and linolenic acids (Jůzlová *et al.* 1996*b*) that can be converted *via* lipoxygenase into eight-carbon compounds (Karahadian *et al.* 1985). The correlation between the contents of these fatty acids and the release of eight-carbon aromatic substances has been described for the mushroom *Lepista nuda* (Noël-Suberville *et al.* 1996). Off-flavors produced due to lipoxygenase activity represent a major problem for many food products (Springett 1993). Methyl ketone (2-nonanone) has been found as an aromatic component of blue cheese formed by *Penicillium roqueforti* (Kinsella and Hwang 1976). Transformation of fatty acids into methyl ketones has also been described in *M. purpureus* (Kranz *et al.* 1992).

Terpenoid compounds (camphene, limonene, thujopsene, 2-methylisoborneol, β-caryophyllene, geosmin, β-cubebene and δ-cadinene) have already been found in fungi of the genera *Penicillium* and *Aspergillus* (Jolivet and Belin 1993; Sunesson *et al.* 1995; Karahadian *et al.* 1985; Börjesson *et al.* 1992). Our strain of *M. purpureus* produces large quantities of polyketide pigments and it has been reported (Jůzlová *et al.* 1996*a*) that some *Monascus* strains can produce monacolin. Although the terpene biosynthesis differs from the biosynthesis of polyketides, the starting point is the same: acetyl-coenzyme A. The correlation between polyketide and terpene biosynthesis has been observed in the case of *Aspergillus flavus* strains producing both aflatoxin (oligoketide) and sesquiterpenes (Zeringue *et al.* 1993); the atoxigenic *A. flavus* strain did not form sesquiterpenes. On the basis of these results, it would be very interesting to compare the production of volatile terpenoid compounds of pigment producing and non-producing (albino) strains of *M. purpureus*. Geosmin and 2-methylisoborneol are also known as components that may impart musty, earthy odors to the enviroment, for example fish exposed to them become tainted and unacceptable for consumers (Jeon 1993). This could cause troubles also in meat products colored by a *Monascus* preparation.

The development of fruity aroma, described as pineapple-, apple- or pear-like, is associated with formation of ethyl esters (Paillard 1990). Samples with fruity flavor were found to contain high levels of ethanol whose formation may be responsible for the enhanced esterification of some free fatty acids. It is surprising that although *Monascus* samples possessed apple-like odor, they contained only relatively small portion of esters among the identified volatiles. This can be caused either by the method of volatiles separation (the relative amount of esters depends on the separation method and it is about 2.5 times higher in the case of headspace GC than in the case of distillation; Paillard 1990) or by the fact that apple odor is composed from approximately 212 compounds (Paillard 1990). Among the apple flavor compounds are esters, alcohols, aldehydes, ketones, ethers, hydrocarbons and terpenes and some of them are same as for *Monascus* volatiles (*e.g.* acetaldehyde, 1-propanol, 2-methyl-1-butanol, 1-pentanol, 2-heptanone, 1-phenylethanol and α-farnesene).

Although the dose of *Monascus* extract or powder added to meat is usually low (0.5–1 g per kg of meat) (Fabre *et al.* 1993), its flavor could affect the final product.

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REFERENCES

BÖRJESSON T., STÖLLMAN U., SCHNÜRER J.: Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal strains. *Appl.Environ.Microbiol.* 58, 2599–2605 (1992).

ENGAN S., pp. 93–165 in J.R.A. Pollock (Ed.): *Brewing Science*, Vol. 2. Academic Press, London 1981.

FABRE C.E., SANTERRE A.L., LORET M.O., BABERIAN R., PAREILLEUX A., GOMA G., BLANC P.J.: Production and food application of the red pigments of *Monascus ruber*. *J.Food Sci.* 58, 1099–1110 (1993).

HESSELTINE C.W.: A millenium of fungi, food and fermentation. *Mycologia* 57, 149–197 (1965).

JEON I.J.: Undesirable flavors in dairy products, pp. 130–134 in M.J. Saxby (Ed.): *Food Taints and Off-Flavours*. Blackie Academic & Professional, an imprint of Chapman & Hall, Glasgow 1993.

JOLLIVET N., BELIN J.-M.: Comparison of volatile flavor compounds produced by ten strains of *Penicillium camemberti* THOM. *J.Dairy Sci.* **76**, 1837–1844 (1993).

JŮZLOVÁ P.: Biosynthesis of pigments by the fungus *Monascus purpureus*. (In Czech) PhD Thesis. Institute of Chemical Technology, Prague 1994.

JŮZLOVÁ P., MARTÍNKOVÁ L., LOZINSKI J., MACHEK F.: Ethanol as substrate for pigment production by the fungus *Monascus purpureus*. *Enzyme Microb.Technol.* **16**, 996– 1001 (1994).

JŮZLOVÁ P., MARTÍNKOVÁ L., KŘEN V.: Secondary metabolites of the fungus *Monascus*. *J.Industr.Microbiol.* **16**, 163–170 (1996*a*).

JŮZLOVÁ P., ŘEZANKA T., MARTÍNKOVÁ L., KŘEN V.: Identification of long-chain fatty acids from *Monascus*, the producer of red pigments. *Phytochemistry* **43**, 151–153 (1996*b*).

KARAHADIAN C., JOSEPHSON D.B., LINDSAY R.C.: Volatile compounds from *Penicillium* sp. contributing musty-earthy notes to Brie and Camembert cheese flavors. *J.Agric.Food Chem.* **33**, 339–343 (1985).

KINSELLA J.E., HWANG D.: Biosynthesis of flavors by *Penicillium roqueforti*. *Biotechnol.Bioeng.* **18**, 927–938 (1976).

KRANZ C., PANITZ C., KUNZ B.: Biotransformation of free fatty acids in mixtures to methyl ketones by *Monascus purpureus*. *Appl.Microbiol.Biotechnol.* **36**, 436–439 (1992).

LIN C.F.: Isolation and cultural conditions of *Monascus* sp. for the production of pigments in a submerged shaken culture. *J.Ferment.Technol.* **51**, 407–414 (1973).

LOTONG N., SUWANARIT P.: Fermentation of ang-kak in plastic bags and regulation of pigmentation by inital moisture content. *J.Appl.Bacteriol.* **68**, 565–570 (1990).

NOËL-SUBERVILLE C., CRUZ C., GUINBERTEAU J., MONTURY M.: Correlation between fatty acid content and aromatic compound release in fresh blewit (*Lepista nuda*). *J.Agric.Food Chem.* **44**, 1180–1183 (1996).

PAILLARD N.M.M.: The flavour of apples, pears and quinces, pp.1–41 in I.D. Morton, A.J. MacLeod (Eds): *Food Flavours. Part C: The Flavour of Fruits*. Elsevier 1990.

SPRINGETT M.B.: Formation of off-flavours due to microbiological and enzymic action, pp. 244–255 in M.J. Saxby (Ed.): *Food Taints and Off-Flavours*. Blackie Academic & Professional, an imprint of Chapman & Hall, Glasgow 1993.

SUNESSON A.-L., VAES W.H.J., NILSSON C.-A., BLOMQUIST G., ANDERSSON B., CARLSON R.: Identification of volatile metabolites from five fungal species cultivated on two media. *Appl.Environ.Microbiol.* **61**, 2911–2918 (1995).

ZERINGUE H.J. Jr., BHAINAGAR D., CLEVELAND T.E.: $C_{15}H_{24}$ volatile compounds unique to aflatoxigenic strains of *Aspergillus flavus*. *Appl.Environ.Microbiol.* **59**, 2264–2270 (1993).