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On the Occurrence of *N*-Methyl-*N*-formylhydrazones in Fresh and Processed False Morel, *Gyromitra esculenta*

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Nine volatile *N*-methyl-*N*-formylhydrazones were identified from fresh false morel mushrooms, *Gyromitra esculenta*, at an average combined level of 57 mg/kg. Using high-resolution glass capillary GLC, a method was developed for the control of hyrazone residues in false morel products. Investigation was made of the decrease of levels of hydrazones during boiling and drying processes and of the effect of process conditions on the level of toxin residues. After prolonged drying, the level of hydyrazone residues fell below 3 mg/kg of dried mushroom, and a boiling time of 10 min was required to reduce the level below 1 mg/kg. The results were compared with measured values of the toxicity of the main component of the residues, acetaldehyde *N*-methyl-*N*-formylhydrazone, and with an estimation of the suitability of false morel as an edible mushroom.

False morel, *Gyromitra esculenta* (Pers.) Fr., is a widely distributed spring mushroom eaten dried or boiled in many countries as a choice edible mushroom. Commercial products are also prepared from this mushroom. Fresh false morel is highly poisonous, and raw or incompletely processed mushrooms have caused many fatal cases of food poisoning (Mlodecki et al., 1962; Franke et al., 1967). The toxicity of false morel has been reported to result not from the so-called helvella acid (Boehm and Kuelz, 1885), but from a compound named gyromitrin. In its structure, gyromitrin is a hydrazone, i.e., acetaldehyde *N*-methyl-*N*-formylhydrazone (acetaldehyde MFH) (List and Luft, 1968a). In addition to acetaldehyde MFH, three of its higher homologues have also been isolated from the steam distillate of *Gyromitra esculenta*. These homologues have been shown to be compounds **4**, **5**, and **6** in Table I, (Pyysalo, 1976a).

The toxicity of acetaldehyde MFH has recently been shown to be for rabbits about LD = 70 mg/kg, for rats about 320 mg/kg, and for chickens over 400 mg/kg (Mäkinen et al., 1977). In a 90-day "short-term" test, peroral administration of acetaldehyde MFH was employed and "no-effect" values obtained were 0.05 mg kg^{-1} day^{-1} for chickens and 0.5 mg kg^{-1} day^{-1} for rabbits (Niskanen et al., 1976). In preliminary animal tests the known higher MFH homologues **4**, **5**, and **6** were shown to have lower values of acute toxicity for rabbits than did acetaldehyde MFH itself (Pyysalo, 1975).

As considerable residues of acetaldehyde MFH have been detected in dried and boiled false morel, the suitability of the mushroom as a commercial food product has been doubted (Gray, 1972; List and Sundermann, 1974; Schmidlin-Meszaros, 1974, 1975; Pyysalo, 1976b). On the other hand, the toxic properties of acetaldehyde MFH and the amount of MFH homologues in false morel have not been known until recently, and the reported results of quantitative estimations of acetaldehyde MFH differ considerably from the results obtained in the present work.

This report is part of a program of research dealing with the toxicity of false morel. The aim has been to develop a sensitive method for estimation of *N*-methyl-*N*-formylhydrazone (MFH) which would be suitable for process control and to clarify the processing conditions necessary to eliminate hydrazone residues or reduce their amount to acceptable levels on the basis of results from animal experiments.

EXPERIMENTAL SECTION

Materials and Processing Conditions. Fresh, fully grown false morel mushrooms, pileus diameter approximately 5 cm, were used. The mushrooms were collected in early June in southern Finland. Drying tests were carried out in dry outdoor air (15–20 °C), in a heat-controlled chamber supplied with through-flow draught and in a commercial fruit and vegetable drier, in which a strong controlled-temperature air current was passed through the mushrooms. In boiling tests a 50-g sample was placed in boiling water, after which boiling resumed in 30 s. The moment of resumption of boiling was taken as zero. After boiling, the mushrooms were filtered and rapidly rinsed once with running tap water.

Quantitative Estimation of *N*-Methyl-*N*-formylhydrazones Using GLC. A known amount of compound **5** was added to the mushrooms as an internal standard and was allowed to adsorb into the mushroom material in a Soxhlet apparatus. MFH compounds were extracted for

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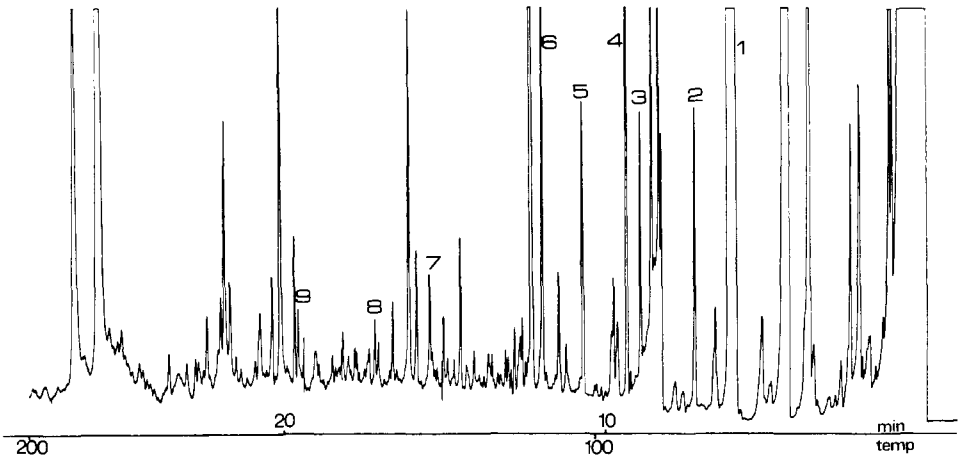

Figure 1. Gas chromatogram of ether extract of fresh false morel, obtained using a 50-m FFAP glass capillary column, showing peaks caused by volatile *N*-methyl-*N*-formylhydrazones.

Table I. *N*-Methyl-*N*-formylhydrazones Identified from False Morel and Their Abundance

MFH compd	Abundance, mg/kg
1, acetaldehyde	49.9
2, propanal	1.0
3, butanal	0.6
4, 3-methylbutanal	2.2
5, pentanal	0.8
6, hexanal	1.4
7, octanal	0.2
8, *trans*-2-octenal	0.6
9, *cis*-2-octenal	0.3
Total	57.0

15 h using ether saturated with water. The ether was dried with anhydrous sodium sulfate and concentrated to 0.5 mL in an apparatus as described by Honkanen and Karvonen (1963). The ether concentrate was injected to a gas chromatograph under the conditions described in Figure 1. The column was a 50-m FFAP glass capillary column, i.d. 0.25 mm, the inner surface of which was inactivated by the method of Blomberg (1975). The column was slightly basic (Grob and Grob, 1971). The magnitudes of the GLC signals caused by MFH homologues were compared with that of the internal standard, and taking into account the GLC responses measured for pure synthetic compounds, the amounts of MFH homologues were calculated.

Identification of *N*-Methyl-*N*-formylhydrazones. Compounds were identified from the ether extract obtained from false morels using the GLC–MS method, incorporating the FFAP column described above. *N*-Methyl-*N*-formylhydrazones were synthesized analogically by the method of List and Luft (1968a), and the structures of the synthetic products were determined using ^{1}H NMR, ^{13}C NMR, IR, and mass spectroscopy (Pyysalo, 1976a). The synthetic hydrazones were shown to be identical with those isolated from false morel, on the basis of their identical mass spectra (Pyysalo and Honkanen, 1976) and similar retention times in GLC using the FFAP column described above and the program outlined in Figure 1.

RESULTS

In addition to the known MFH compounds **1**, **4**, **5**, and **6** (Table I), some previously unrecognized MFH homologues (**2**, **3**, **7**, **8**, and **9**) were also detected from false morel. The structures of these compounds and their abundance in fresh mushrooms are shown in Table I.

Drying Tests. The average dry matter content of fresh false morel was 7.2% by weight. Table II shows the reduction in MFH content taking place during drying of mushrooms outdoors in the sun at an average temperature of 20 °C, which is the normal treatment in household preparation. Drying tests were also made at 30, 40, 50, and 60 °C in an incubator fitted with through-flow draught and in a warm air drier. The results showed that the rise in temperature did not cause a rapid decrease in the content of MFH compared with the values in Table II. In all these experiments, despite high dry weight values, the mushrooms still contained high levels of MFH. Table III shows

Table II. Amounts of *N*-Methyl-*N*-formylhydrazones during Drying of Fresh False Morel in Outdoor Air[a]

Time of drying, days	Weight of sample, g	Total MFH, mg	% of the orig MFH	MFH per kg, mg	Dry matter, %
0	50	2.85	100	57.0	7.2
1	36	0.90	31.6	25.0	10.0
2	18.4	0.70	24.6	38.0	19.6
5	7.8	0.35	12.3	44.9	46.2
10	4.7	0.30	10.1	63.8	76.6
14	5.2	0.03	1.1	5.8	69.2
180[a]	3.9	0.01	0.35	2.6	92.3

[a] Storage at 20 °C.

Table III. Amounts of *N*-Methyl-*N*-formylhydrazones during Drying of Fresh False Morel at 50 °C

Time of drying, min	Weight of sample, g	Total MFH, mg	% of the orig MFH	MFH per kg, mg	Dry matter, %
0	50	2.85	100	57.0	7.2
10	42	2.35	82.5	56.0	8.6
30	27	1.48	51.9	54.8	13.3
75	17.5	1.25	43.9	71.4	20.6
120	5.9	0.95	33.3	161.0	61.0
150	4.1	0.59	20.7	143.9	87.8
140	3.7	0.23	8.1	62.2	97.3

Table IV. Amounts of *N*-Methyl-*N*-formylhydrazones in Boiled False Morel (100 g of mushrooms/300 mL of water, pH 7.8)

Boiling time, min	Weight of sample, g	Total MFH, mg	% of the orig MFH	MFH per kg, mg
0	43.8	0.16	2.8	3.65
1	46.7	0.15	2.6	3.21
2	45.0	0.09	1.6	2.00
4	41.7	0.08	1.4	1.92
10	39.2	0.03	0.53	0.77
2 × 5	43.8	0.028	0.49	0.64

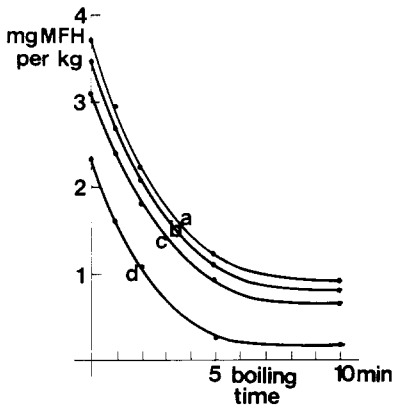

Figure 2. Amounts of *N*-methyl-*N*-formylhydrazones per kilogram of boiled mushrooms remaining during boiling of 100 g of false morel in (a) 200 mL, (b) 300 mL, (c) 400 mL, and (d) 600 mL of water, pH 7.8.

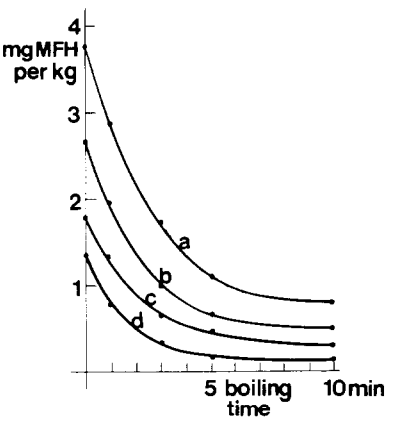

Figure 3. Amounts of *N*-methyl-*N*-formylhydrazones per kilogram of boiled mushrooms after boiling 100 g of false morel in 300 mL of water at pH values of (a) 8.0, (b) 6.5, (c) 5.0, and (d) 3.5.

the values obtained from mushrooms dried at 50 °C. The values are typical of these drying experiments.

Boiling Tests. Boiling conditions have a significant effect on the rate of decrease of MFH levels in false morel. Table IV shows the amounts of MFH remaining after boiling 100 g of false morel in 300 mL of water (pH 7.8) for different lengths of time (original concentration, 57 mg of MFH/kg). The effect of the quantity of boiling water on the rate of disappearance of MFH is shown in Figure 2. In addition to an increase of the amount of boiling water, reduction of its pH also speeds up the disappearance of MFH. Figure 3 shows residual MFH levels during boiling of 100-g samples of mushrooms in 300 mL of water at four different pH values.

Relative Concentrations of *N*-Methyl-*N*-formylhydrazones in Processed Mushrooms. Amounts of different MFH homologues occurring in fresh false morel are shown in Table I. During drying the relative amounts of the homologues remain approximately the same, so that acetaldehyde MFH is still the main component. In mushrooms dried for 10 days (Table II), amounts of the compounds **1**–**9** were 52.4, 1.3, 0.4, 5.2, 2.6, 1.3, 0.2, 0.2, and 0.2 mg/kg (total MFH 63.8 mg/kg). After 4 min of boiling (Table IV), the amounts of compounds **1**–**9** were 1.23, 0.02, 0.03, 0.03, 0.02, 0.22, 0.13, 0.22, and 0.02 mg/kg (total 1.92 mg/kg). It has been noticed that in the steam distillate from the boiling process the relative amounts of the different MFH compounds were significantly different from those above: compounds **1**, **4**, **5**, and **6** were present in the ratio 0.9:3.3:0.1:0.6 (Pyysalo, 1975).

DISCUSSION

The total content of the volatile MFH compounds measured in the present work by GLC differs considerably from the value obtained by calculating the potassium iodate reducing materials as acetaldehyde MFH. The latter value may rise in fresh false morels as high as 1600 mg/kg (List and Luft, 1968b). It seems likely that false morel also contains other MFH compounds than those here identified by the GLC–MS method. However, in preliminary animal tests in which two 2-kg rabbits were fed with the juice from 0.5 kg of fresh false morel containing 25 mg of MFH by GLC estimation, no effects attributable to toxicity were observed. This indicates that acetaldehyde MFH (LD_{50} = 70 mg/kg) is the main toxic agent in false morel and that the total MFH content and the acetaldehyde MFH content measured in this work by GLC correlates well with the toxicity level of fresh mushrooms.

By contrast, the press juice fed to the rabbits contained 350 mg of potassium iodate reducing materials, which in the form of MFH would certainly have caused by rapid death of the test animals. A different question is whether the toxicity results from the hydrazones themselves or from products of their metabolism, possibly methylhydrazine (Schmidlin-Meszaros, 1975; Gray, 1972).

Several conclusions can be drawn from the results of the processing tests. MFH compounds do not evaporate quickly from false morel, and it is important that the mushrooms be stored in open surroundings until used, as the poison level continues to fall even after the dry matter content reaches a near-constant value (Table II). In rapid high-temperature drying there is a danger that, despite high dry-matter content, considerable residues of MFH remain (Table III).

When boiling is used, a minimum of 3 L of water per kilogram of mushrooms should be used. During heating and the first moments of boiling, most of the MFH is destroyed, but only after 10 min of boiling does it fall below 1% of the original value (Table IV). The efficiency of the boiling process can be increased by boiling twice, with a change of water.

The toxicity of acetaldehyde *N*-methyl-*N*-formylhydrazone, the main component of hydrazone compounds in false morel, has been investigated in animal tests. On the basis of safety evaluation for toxic compounds, certain preliminary estimates can be made concerning the toxicity of false morel food products (Goldberg, 1975). In the 90-day "short-term" test, the most sensitive animal was the chicken, with a "no-effect" value of 0.05 mg of acetaldehyde MFH per kilogram per day. For a 70-kg subject the corresponding value would be 3.5 mg. Using a safety factor of 100, the maximum permissible amount of acetaldehyde MFH per day would be 0.035 mg. This amount is contained in approximately 5 g of dried false morel, obtained from drying 50 g of fresh mushrooms outdoors for 14 days (Table II). Fresh mushrooms (100 g) boiled for 10 min in 300 mL of tap water give approximately 40 g of boiled mushrooms, which contain 0.03 mg of MFH (Table IV). Although the LD_{50} value and the short-term "no-effect" value of acetaldehyde MFH are known, no acceptable ADI value can be given until the products of MFH metabolism, possibly even more toxic than the parent compound, have been studied. The approximate value reached above does not take into account the fact that the MFH content of fresh false morel may vary according to growth conditions and degree of maturity. On the other hand, dried and boiled mushrooms are normally

further heated before consumption, upon which the amount of residues again decreases.

The observations concerning MFH residues made in this work agree with those of other researchers and confirm that false morel mushrooms should be processed with great care. The method described for the determination of MFH compounds, although precise and suitable for control procedures, requires high gas chromatographic separation efficiency and careful selection of the correct column. The FFAP glass capillary column should be neutral or slightly basic since MFH compounds are not quantitatively determined in acid conditions, leading to the possibility of faulty determinations. However, standards for comparison can be easily prepared from commercial chemicals, although because of their tendency to decompose, the purity of MFH compounds should be repeatedly confirmed, for example, using ^{1}H NMR.

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Physical Stability of Milk Fat Emulsions after Processing as Evaluated by Response Surface Methodology

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Computer analysis of a response surface experimental design was used to evaluate the effect of three variables, homogenization temperature (50, 60, 70 °C), pressure (1000, 1500, 2000 psi), and emulsifier concentration upon physical stability of 25% milk fat emulsions. The first series of emulsions contained nonfat milk solids (NFMS) at 1, 6, and 11% levels, but no commercial emulsifier. The second and third series contained 1% sodium caseinate and 9% NFMS, respectively, plus emulsifier. Emulsifiers and their concentrations in the fat phase were: RG Lecithin at 0, 0.5, and 1.0%; Emplex at 0, 0.05, and 0.1%; and Tween 20 at 0, 0.5, and 1.0%. A small-scale pilot plant homogenizer was used to prepare each emulsion. Stability data for each experiment were analyzed by fitting a Taylor second-order equation for three independent variables. Response surface plots were generated by the computer for emulsion stability as a function of temperature vs. pressure at the selected NFMS or emulsifier level. In each plot there were many combinations of temperature and pressure that would produce the same response. Pressure generally was more important than temperature or emulsifier level in determining stability. However, a variety of curvilinear interrelationships among the variables was evident from the different forms of the curves (rising or falling ridges, bull's-eyes, saddles). A combination of variables can be selected by this procedure to give an optimum or a desired emulsion stability.

Many formulated foods are made by emulsifying liquid fat into an aqueous phase containing proteins, carbohydrates, and other materials. The nutritional and functional properties of such food emulsions are of great importance to the processor and consumer. There is a need to develop improved laboratory and pilot plant procedures to guide the formulation and preparation of new foods and to improve current processing practices. In our previous research (Smith and Dairiki, 1975a,b), techniques were reported to prepare and evaluate the physical stability of model oil-in-water emulsions as influenced by sodium caseinate, nonfat milk solids (NFMS), and different emulsifiers. With more complicated systems, interference occurred between some emulsifiers and the other components in experiments where we varied one ingredient at a time while other components were held "constant". It became apparent that emulsion variables cannot be assumed to act independently as implied by single-factor experiments.

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