Methyl Ketone Formation During Germination of *Penicillium roqueforti*

Tsai Y. Fan, Dong H. Hwang, and John E. Kinsella*

Spores of *Penicillium roqueforti* germinated in a corn steep liquor medium at 30 °C attained maximum germination within 16 h. The dry weight of the spores increased progressively during germination; however, the capacity to form 2-heptanone from octanoic acid diminished and attained a minimum when germ tubes were emerging from the spores. Methyl ketone formation attained the same level as in resting spores when maximum germination was reached and further accelerated as germ tube elongated into mycelium. Octanoic acid (1 mM) inhibited the formation of 2-heptanone by both spores and mycelium. Resting spores were more resistant to octanoate than the germinated spores. This was related to cell wall thickness. 2-Heptanone was metabolized when it was incubated with either resting spores or germinated spores. This activity was influenced by concentration of the substrate and physiological stage of the mold.

The methyl ketones of intermediate chain length (C_5–C_{13}) are the important flavors of mold-ripened cheeses (Patton, 1950; Schwartz and Parks, 1963; Anderson and Day, 1966; Dartey and Kinsella, 1971). The mold *Penicillium roqueforti* which is involved in the ripening of blue cheese can oxidize fatty acids to the corresponding methyl ketones with one less carbon atom (Girolami and Knight, 1955; Gehrig and Knight, 1963; Lawrence, 1965b, 1966; Lawrence and Hawke, 1968; Dartey and Kinsella, 1973a,b). The pathway for the transformation of fatty acids to methyl ketones was outlined by Hawke (1966).

There is controversy in the literature as to whether spores or mycelium account for methyl ketone formation via oxidation of fatty acids. A decline in the production of methyl ketones as spores germinated to mycelium was reported by Gehrig and Knight (1958, 1961, 1963). They concluded that spores were the active agents in the metabolism of fatty acids to methyl ketones. On the other hand, Lawrence and Hawke (1968) and Dwivedi and Kinsella (1974) demonstrated successful production of methyl ketones from fatty acids by vegetative mycelium, whereas resting spores showed very low activity (Lawrence 1965b, 1966). The addition of sugars or amino acids along with fatty acids to suspensions of resting spores enhanced the oxidation of fatty acids with concurrent production of methyl ketones (Lawrence, 1965b). It was speculated that sugar and amino acids activated the resting spores, induced germination, and concurrently stimulated the production of methyl ketone.

Although Lawrence and coworkers (Lawrence, 1965b, 1966; Lawrence and Hawke, 1968; Lawrence and Bailey, 1970) have studied fatty acid oxidation by spores and mycelium, a quantitative comparison of their relative capacities has not been made and neither has the changing capacity been assessed at progressive stages of germination and growth. Such information and factors affecting rate of germination are of practical significance in developing fermentation systems for production of flavor chemicals.

In the present studies, the rate of germination of spores of *P. roqueforti* and their changing capacity to synthesize methyl ketones were determined.

MATERIALS AND METHODS

Cultivation and Harvesting of Spores. Spores of *P. roqueforti* (Midwest Blue Mold Co., Stillwater, Minn.) were grown as surface culture on a medium composed of 1% corn steep liquor (CPC International, Agro, Ill.) and 1% sucrose at 25 °C. After 5 days of undisturbed growth, the spent medium was drained carefully from the incubation flask. Distilled water was added and the flask was shaken vigorously to dislodge the spores. The spore suspension was filtered through a double layer of cheese cloth. The filtrate was further filtered on a Buchner funnel with Whatman No. 1 filter paper. The spores retained on the filter paper were washed twice with distilled water by alternative centrifugation and resuspension. The spores were stored in distilled water at 4 °C and used within 1 month. The concentration of the spores was determined using a hemacytometer.

Germination of Spores. Resting spores of *P. roqueforti* (10^7/ml) were incubated in a germination medium consisting of 2% corn steep liquor and 1.5% sucrose. Streptomycin (0.4 mg/ml) was added to inhibit bacterial contamination. The incubator (New Brunswick Scientific Co., New Brunswick, N.J.) was maintained at 30 °C with rotation speed at 110 rpm. At progressive stages of incubation, flasks were removed from the incubator. The contents of the incubation flask was centrifuged. The pelleted spores were washed twice with distilled water and suspended in 0.03 M potassium phosphate buffer (pH 6.5) for further use. Duplicate aliquots of spore suspension were placed on a hemacytometer and percent germination was determined using light microscopy. Presence of a germ tube equal or longer than the diameter of the spore was the criterion of germination. The length of germ tube was determined by a micrometer attached to the eyepiece of the microscope which was calibrated against a micrometer slide (2 mm divided into 0.01 mm, American Optical Co., Buffalo, N.Y.). Forty germinated spores were measured and the average length was reported.

Determination of Dry Weight. A Millipore filter paper (Millipore Corp., Bedford, Mass.) was preheated in a forced air oven at 85 °C overnight, cooled in a desiccator, and weighed. An aliquot of the suspension of spores or mycelium was filtered through the filter paper, washed with distilled water, and dried in an oven at 85 °C to constant weight. The dry weight of spores or mycelium was determined by the weight difference.

Ultrastructure of Cell Wall. Resting spores, germinated spore, and mycelium were fixed in two changes of 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) overnight at 4 °C, washed three times with the same buffer, and postfixed in 2% OsO4 in distilled water for 2 h. Samples were washed three times with distilled water, stained with 1% uranyl acetate for 30 min, and washed again three times with distilled water. Dehydration

Department of Food Science, Cornell University, Ithaca, New York 14853.

consisted of two changes of 50, 70, 80, and 95% ethanol (15 min at 4 °C), and 100% ethanol (30 min at room temperature). Dehydrated samples were put into two changes of propylene oxide (10 min each) and then into the mixture of Epon and propylene oxide (1:1, v/v) for 5 h. The samples were embedded in Epon (Luft, 1961). Ultrasections were cut on a Sorvall ultramicrotone (Model MT-2), double stained with 1% uranyl acetate and lead citrate, and studied in a Philips EM 300 electron microscope.

Oxidation of Octanoic Acid to 2-Heptanone. The relative ability of spores at different germination stages to produce 2-heptanone from octanoic acid was compared based on either an equal number of original resting spores or an equal weight of spores.

Octanoic acid (3 μmol) was added to 3 ml of spore suspension in phosphate buffer containing 10^7 original resting spores per ml, or 1 mg of spores for the isogravimetric study. The spore suspension was sealed in a screw-capped vial (20 ml capacity, 5.5 cm in height, and 2.5 cm in diameter) and incubated at 30 °C with shaking. At predetermined time intervals, the vials were removed from the incubator for the determination of 2-heptanone concentration.

Metabolism of 2-Heptanone. 2-Heptanone (1.3 or 2.1 μmol) was added to 1 to 3 mg of spores or mycelia in 3 ml of phosphate buffer in screw-capped vials. The vials were incubated at 30 °C with shaking and removed from the incubator every 2 h to determine the residual 2-heptanone concentration.

Determination of 2-Heptanone. Methyl ketones were quantified as their dinitrophenylhydrazone derivatives. 2,4-Dinitrophenylhydrazine (DNPH) reagent (2 g/l. of 2 N HCl) (Lawrence, 1965a) (3 ml) was added to vials immediately after they were removed from the incubator to inactivate the spores and form DNPH derivatives of methyl ketones. DNPH derivatives were twice extracted with 6 ml of hexane 3 h later. The hexane extracts were combined, evaporated to dryness, and redissolved in 0.5 ml of hexane. Standard solutions containing known concentrations of pure 2-heptanone (Aldrich Chemical Co., Milwaukee, Wis.) were formed from DNPH derivatives, extracted with hexane, and concentrated following the same procedures as 2-heptanone produced in vials.

DNPH derivatives were separated by reverse-phase thin-layer chromatography (Dartey and Kinsella, 1973a). Thin-layer plates coated with 0.25 mm of Kiesulguhr G (E. Merck Lab. Inc., Elmsford, N.Y.) were activated in an oven at 110 °C for 30 min and impregnated with a 10% solution of 2-phenoxyethanol (Eastman Chemical Co., Rochester, N.Y.) in acetone. Aliquots of concentrated hexane extract were spotted on the plates which were developed three times in hexane saturated with polyethylene glycol 400. The spot containing the DNPH derivative of 2-heptanone was scraped into a pasteur pipet plugged with glass wool at the tip and the 2-heptanone derivative was eluted with hexane (6 ml). The absorbance of eluates was read at 345 nm. The concentration of 2-heptanone was determined from a standard curve prepared using pure 2-heptanone.

RESULTS

Germination of Spores. The germination pattern of *P. roqueforti* spores was similar to that described for other fungal spores (Gottlieb, 1950; Cochrane, 1960; Allen, 1965). The germination sequence involved initial swelling of the resting spores followed by the formation of germ tubes and subsequent elongation of germ tubes into hyphae and mycelium.

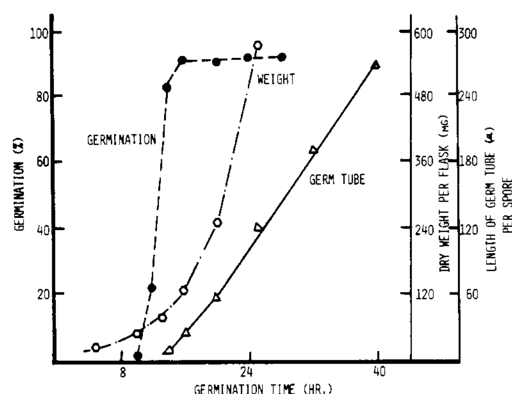

Figure 1. The germination rate (percent) and changes in dry weight and length of germ tube during germination of spores of *Penicillium roqueforti*. The flask contains 10^9 resting spores and 100 ml of germination medium initially and is incubated at 30 °C with shaking.

Resting spores began to swell at approximately 4 h after the spores were inoculated in the germination medium. Between 11 and 12 h after starting the spore incubation, germ-tube emergence was observed (Figure 1). The number of spores with germ tubes increased rapidly and 80% germination was found after 14 h. Maximum germination occurred at 16 h, when germination reached 91% and remained at this level afterward. Elongation of germ tubes maintained a constant rate of 20 μm per h for each spore during 14–40 h of incubation (Figure 1).

The germination process could be arbitrarily divided into five phases: resting spores (0 h), swelling spores (1–10 h), germ-tube emergence stage (11–16 h), germ-tube elongation stage (17 h and afterward), and mycelium. It was difficult to determine at what stage the germ tubes developed into mycelium. To avoid confusion, terms such as "spores germinated for x h" are used herein, although in many occasions these "spores" might better be classified as mycelium.

During germination, the dry weight of the spores increased due to the absorption of exogenous nutrients from the medium. The increase was very slow during the swelling phase, but became very rapid during germ-tube emergence and elongation (Figure 1).

The cell wall of resting spore as revealed by electron microscopy consists of four layers (W1, W2, W3, and W4). The outermost layer (W1) ruptured and separated as the resting spore swelled. The cell wall of the germ tube was much thinner than that of the spore and had only electron transparent layer (W3). The outermost layer (W1) in the resting spore was not seen in the mycelial cell wall (Figures 2a–c).

Formation of 2-Heptanone during Spore Germination. The relative capacity of *P. roqueforti* spores at various stages of germination to produce 2-heptanone via the oxidation of octanoic acid was measured in the present study.

In the first experiment, the number of resting spores was held constant initially while the germination time varied. Since the dry weight of the spores increased as germination progressed, a higher cell mass was formed for the same number of original resting spores at later stages of germination. The physiological stage of the spore clearly affected the rate of 2-heptanone formation (Figure 3). Resting spores showed appreciable activity in the production of 2-heptanone. However, as spores germinated, their activity was reduced despite their higher cell mass. The amount of 2-heptanone produced by spores at the swelling and germ-tube emergence stages was considerably

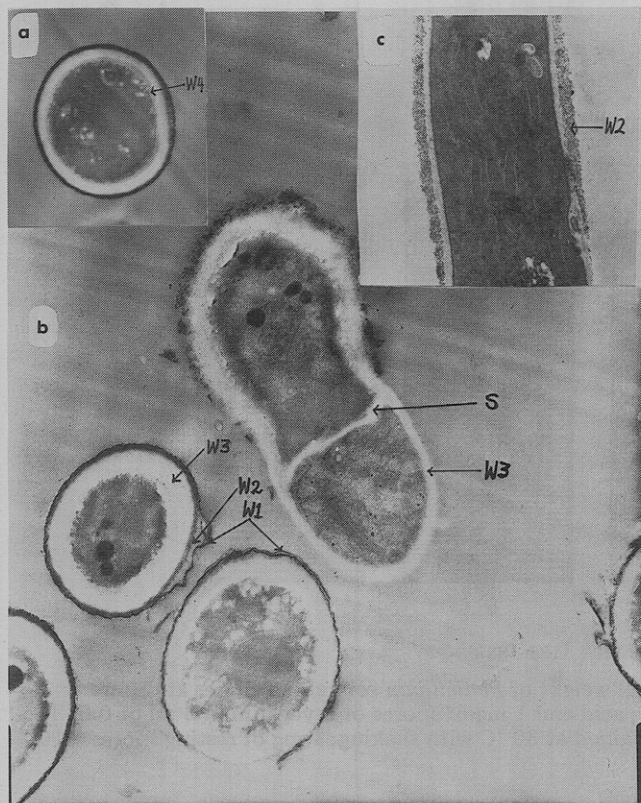

Figure 2. (a) Thin section through resting spores of *P. roqueforti*; W4, inner wall layer; ×7417; (b) thin sections through swelling spore and germinated spore; W1, electron dense outmost layer; W2, second wall layer; W3, electron transparent layer; S, septum; ×8750; (c) thin section through mycelium; W2, second wall layer; ×4333 (courtesy of Professor L. Hood, Cornell University).

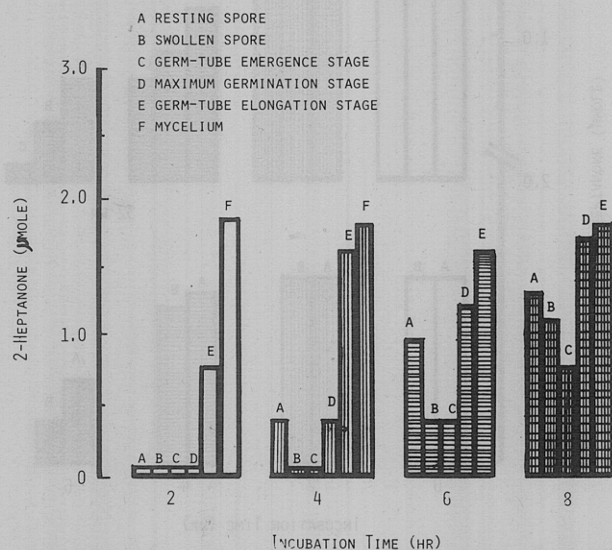

Figure 3. Production of 2-heptanone from octanoic acid by *Penicillium roqueforti* spores at different stages of germination. Each incubation contained the equivalent of 10^7 initial resting spores per ml and 1 mM of octanoic acid in 0.03 M potassium phosphate buffer at pH 6.5. Total incubation volume was 3 ml. The incubator was maintained at 30 °C with shaking.

less than that produced by resting spores. Also, their lag periods were lengthened. 2-Heptanone productivity reached a minimum level when spores at germ-tube emergence stage were used. Once the maximum germination stage was attained, the ability of spores to produce 2-heptanone was restored to a level slightly exceeding that

Table I. Production of 2-Heptanone from Different Concentrations of Octanoic Acid by *Penicillium roqueforti* at Different Stages of Germination[a]

[Octanoic acid], μmol	μmol of 2-heptanone produced at spore germination age: Resting spore	24 h	32 h
3	2.20	2.40	2.03
6	4.14	3.23	1.30
9	5.94	0.73	0.36
12	7.14	0.28	0.26
15	6.54	0	0

[a] The incubation contained indicated amounts of octanoic acid and 1 mg of spore or mycelium in 3 ml of 0.03 M potassium phosphate buffer at pH 6.5. The incubator was maintained at 30 °C with shaking. Incubation time for 2-heptanone production was 5 h; 1 mg of resting spores = 10^8 spores.

of resting spores. Spores after the germ-tube elongation stage were extremely active in the metabolism of octanoic acid to 2-heptanone.

A comparison of the relative capacity of equal weights of spores at different stages of germination to generate 2-heptanone was made (Figure 4). Swelling spores and spores at the germ-tube emergence stage were not included in the figure, since these spores had higher dry weights but consistently exhibited lower 2-heptanone productivity than observed with resting spores. Spores at the early germ-tube elongation stage (20 h germination) showed the lowest efficiency in the formation of 2-heptanone per unit weight of spores, while resting spores and spores germinated for 24, 28, and 32 h had comparable rates of 2-heptanone production.

The relative abilities of resting spores and spores germinated for 24 and 32 h to produce 2-heptanone from octanoic acid at increasing concentrations were compared using equal weights of spore preparations (Table I). Octanoic acid appeared to inhibit the production of 2-heptanone at various germination stages. When the concentration of octanoic acid was doubled from 3 to 6 μmol, resting spores were able to produce twice as much 2-heptanone in response to the increased concentration of octanoic acid. However, a continued increase in the concentration of octanoic acid caused a progressive diminution in heptanone production so that ultimately resting spores produced less 2-heptanone from 15 μmol of octanoic acid than from 12 μmol of octanoic acid. A similar phenomenon was observed with spores germinated for 24 and 32 h. The concentrations of octanoic acid above which an increasing concentration of substrate resulted in reduction of 2-heptanone production were 6 and 3 μmol per mg for 24 and 32 h germination spores, respectively. Resting spores were most resistant to the inhibition by octanoic acid.

Metabolism of 2-Heptanone. Results (Figure 4) showed that prolonged incubation during the formation of 2-heptanone from octanoic acid resulted in a reduced concentration of 2-heptanone. When known concentrations of 2-heptanone were incubated in phosphate buffer alone, no loss of 2-heptanone was observed, thus eliminating the possibility that 2-heptanone disappeared through evaporation during incubation. It was likely that 2-heptanone was further metabolized by spores of *P. roqueforti*. Studies were undertaken to determine the rate of the metabolism of 2-heptanone with the results shown in Figures 5 and 6.

The rate of utilization of 2-heptanone was influenced by both the physiological stage of the spores and the initial

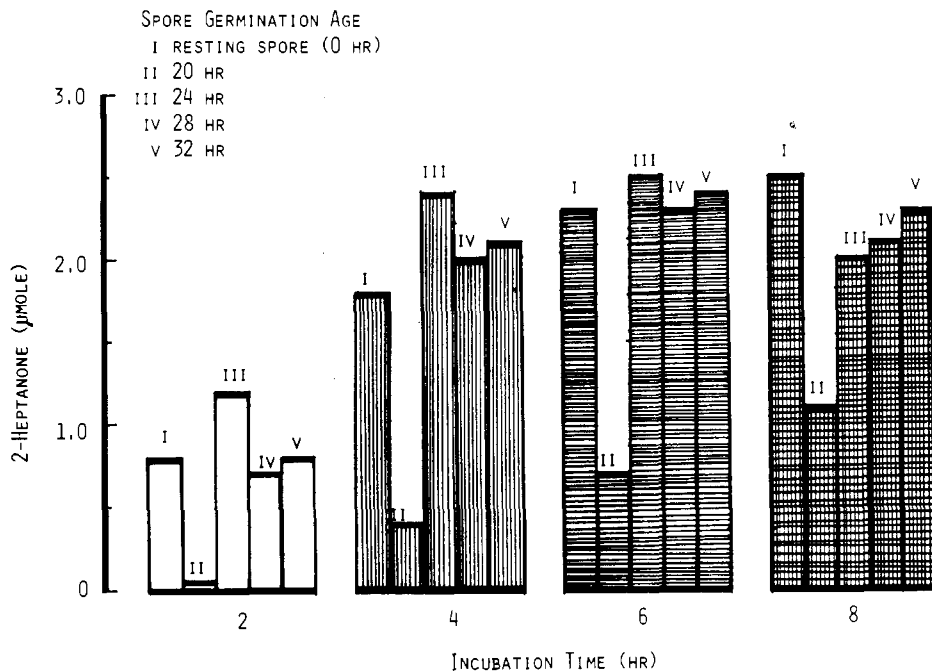

Figure 4. Production of 2-heptanone from octanoic acid by equal weight of *Penicillium roqueforti* spores at different germination stage. The incubation contained 3 μmol of octanoic acid and 1 mg of spores or mycelium in 3 ml of 0.03 M potassium phosphate buffer at pH 6.5. The incubator was maintained at 30 °C with shaking; 1 mg of resting spores = 10^8 spores.

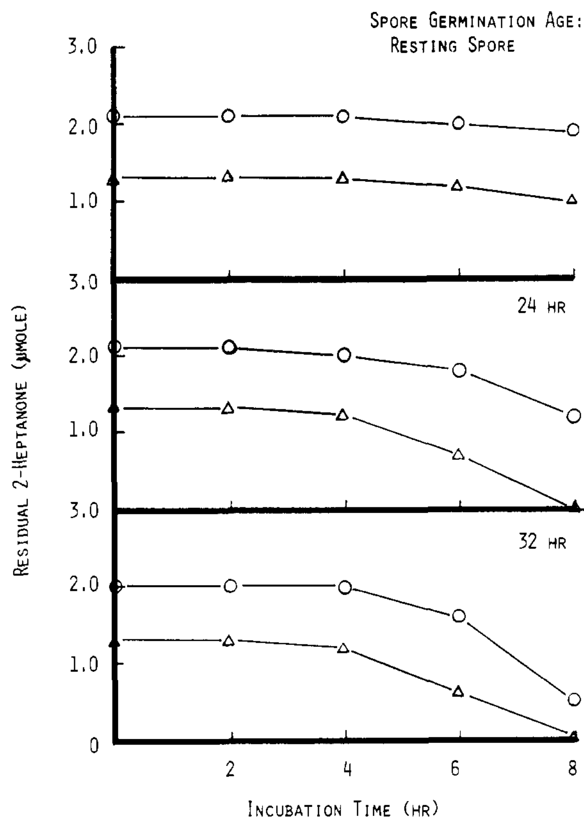

Figure 5. Disappearance of 2-heptanone during the incubation with *Penicillium roqueforti* at different germination stages. The incubation contained indicated amounts of 2-heptanone initially and 1 mg of spore or mycelium in 3 ml of 0.03 M potassium phosphate buffer at pH 6.5. The incubator was maintained at 30 °C with shaking.

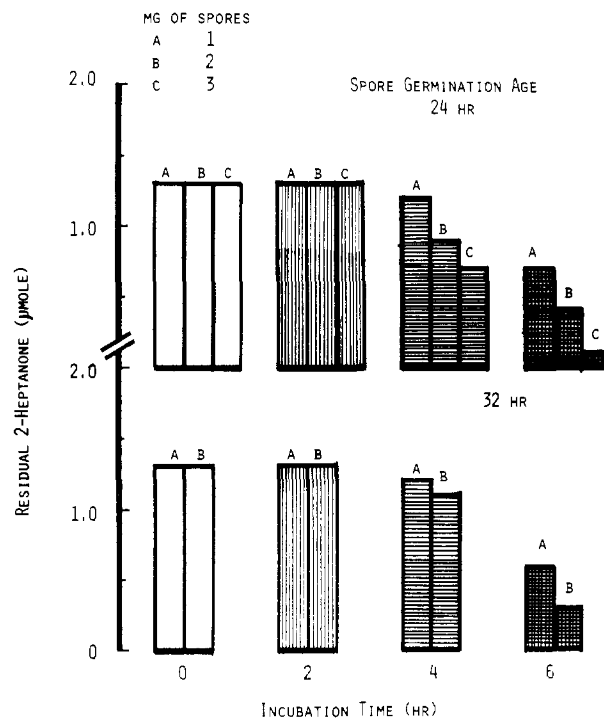

Figure 6. Disappearance of 2-heptanone during the incubation with different dry weight levels of *Penicillium roqueforti*. The incubation contained 1.3 μmol of 2-heptanone initially and 1 to 3 mg (A,B,C) of spores or mycelium in 3 ml of 0.03 M potassium phosphate buffer at pH 6.5. The incubation was maintained at 30 °C with shaking.

concentration of 2-heptanone (Figure 5). Resting spores metabolized 2-heptanone extremely slowly. A lag period of 4 h was observed and after 8 h of incubation, 1.9 and 1.0 μmol of 2-heptanone still remained from the initial 2.1 and 1.3 μmol, respectively. Germinated spores showed considerably higher activity toward the metabolism of 2-heptanone. A lag period of 4 h was still observed; however, 2-heptanone was rapidly reduced afterward. At an initial concentration of 1.3 μmol, 2-heptanone disap-

peared completely after 8 h of incubation with either 24 or 32 h germination spores. At a higher initial concentration (2.1 μmol), 2-heptanone concentration decreased more slowly. Spores germinated for 32 h seemed to metabolize 2-heptanone faster than spores germinated for 24 h.

The metabolic rate was also dependent on the dry weight of spores inoculated (Figure 6). Increasing the amount of spores increased the rate of 2-heptanone disappearance. This was true for both spores germinated for 24 and 32 h.

DISCUSSION

From the results of present investigations, it may be concluded that both resting spores and mycelium of *P. roqueforti* are capable of oxidizing fatty acids to methyl ketones (Figures 3 and 4). This conclusion is consistent with the literature reports that methyl ketones can be produced by either spores (Gehrig and Knight, 1958, 1961, 1963; Lawrence, 1965b, 1966; Dartey and Kinsella, 1973a,b) or mycelium (Lawrence and Hawke, 1968; Dwivedi and Kinsella, 1974). The significance of this report is the demonstration that both resting spores and mycelium seemed equally efficient in the conversion of fatty acids to methyl ketones.

Gehrig and Knight (1958, 1961, 1963) reported the failure of mycelium of *P. roqueforti* to oxidize fatty acids and form methyl ketones. However, it should be noted that *P. roqueforti* is sensitive to the presence of a high concentration of fatty acids (Lawrence, 1966; Lawrence and Hawke, 1968). Extended lag periods were encountered with increasing concentrations of fatty acids (Lawrence, 1966; Lawrence and Hawke, 1968). Between the two mycelium was less resistant to the toxicity of fatty acids than resting spores (Table I). The ratio of concentration of fatty acids to dry weight of spores is a decisive factor in determining the ability of *P. roqueforti* to oxidize fatty acids. In the present study, 15 μmol of octanoic acid completely prevented the formation of 2-heptanone for at least 5 h by 1 mg of spores of *P. roqueforti* which had been previously germinated for 24 and 32 h (Table I). No quantitative data concerning the dry weight of mycelium were described in the works of Gehrig and Knight (1963). However, it was obvious that a high concentration of octanoic acid (20 μmol) might be responsible for the failure of 2-heptanone production. Furthermore, an incubation period of only 2 h in their study would be insufficient to allow 2-heptanone production since it was still in the lag period where no methyl ketone formation could be expected. On the other hand, Lawrence and Hawke (1968) successfully oxidized octanoic acid to 2-heptanone by mycelium of *P. roqueforti*, when large amounts of mycelium (10 mg) were used to incubate with a relatively low concentration of octanoic acid (3 μmol). Thus, whether mycelium is capable of oxidizing fatty acids to methyl ketones seemed to be dictated by whether the concentration ratio of mycelium to fatty acid is favorable.

During germination, the metabolic activity of spores increases tremendously. Oxygen consumption is markedly increased (Caltrider and Gottlieb, 1963) and the activity of enzymes intensifies (Caltrider et al., 1963; Gottlieb and Caltrider, 1963; Ohmori and Gottlieb, 1965; Barash, 1968). However, the capacity of 2-heptanone formation by *P. roqueforti* was apparently reduced during spore germination. The lack of 2-heptanone production is probably attributable to the more complete oxidation of the octanoic acid by the high metabolic activity of germinating spores.

The ultrastructural changes in the morphology of the spores during germination revealed that during the swelling phase, the outer layers of spore walls disintegrated and gradually disappeared. The newly formed germ tubes had only a single layer of cell wall (Figure 2a–c). The ultrastructural changes were similar to those reported by Martin et al. (1973) during the germination of *Penicillium notatum*. These changes may at least partly explain the differential toxicity of fatty acids to spores at progressive stages of germination. Thus, the thick-walled spores were quite resistant to inhibition by fatty acids whereas the germinating spores were susceptible. Conceivably the thin cell wall of the germ tube was much more permeable to fatty acids which thereby entered the cells and impaired overall metabolism.

Methyl ketones produced from the oxidation of fatty acids were further metabolized by *P. roqueforti* (Figures 5 and 6). It was likely that methyl ketones were reduced to the corresponding secondary alcohols as observed by Jackson and Hussong (1958) and Anderson and Day (1966). Several factors which influenced the metabolic rate of 2-heptanone by *P. roqueforti* have been identified in the present study. Changes in the physiological stage of the mold, the dry weight of the mold, the initial concentration of 2-heptanone, and incubation time all lead to the changes in the rate of 2-heptanone disappearance.

The results of the present study not only demonstrated the relative activity of spores and mycelium in the production of methyl ketones, but also provided some practical implications concerning the flavor production during blue cheese ripening. When spores germinate and develop into vegetative mycelium, there is an increase in the dry weight of the cells. A larger amount of vegetative cells inevitably produced a larger quantity of flavor compounds; however, they also metabolized methyl ketones faster. On the other hand, the resting spores were characterized by their high resistance to the toxic effects of fatty acids, their comparable efficiency in the production of methyl ketones, and their limited metabolism of methyl ketones.

In the design of the cheese ripening process, careful consideration should be given to the balance of production and metabolism of methyl ketones in order to know how to manipulate germination and sporulation of the mold to obtain best flavors in the shortest ripening time. In cases where it is desirable to produce methyl ketones in the fermentation tank as a source of artificial flavor such understanding of the factors governing the production and metabolism of methyl ketones is important in order to develop optimum conditions to obtain the maximum yield of flavor compounds.

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Polyphenols in Golden Delicious Apple Juice in Relation to Method of Preparation

J. Van Buren,*[1] L. de Vos, and W. Pilnik

Apple juices prepared by conventional and enzymatic procedures were examined for phenolic materials and color before and after storage. Measurement of both the total phenolic material and the phenolic material precipitated by formaldehyde was supplemented by specific measurement of chlorogenic acid and flavonol glycosides, as well as inspection of chromatograms for flavans. The total phenolics ranged from 25 mg/100 ml in juices from well-oxidized pulps to 150 mg/100 ml in juices made with minimal enzymatic oxidation. Chlorogenic acid and considerable flavonoids were easily extracted into the juice, but they were also lost during the pre-pasteurization period. On the other hand, flavonol glycosides went into the juice slowly, were relatively stable in unpasteurized juices, and were lost during storage, particularly in concentrates. Enzymatic oxidation of apple pulp, presumably by polyphenol oxidase, caused almost complete loss of chlorogenic acid and flavans, but had little effect on flavonol glycosides. Treatment with pectolytic enzymes before pressing led to increased extraction of nonflavonoid Folin-Ciocalteu reactive materials into juice. Reducing materials increased during storage of juice concentrate. Observations of color changes in stored juices indicate that brown pigment development was associated with flavonoids.

The polyphenolic materials of apple juice are of particular interest because the color and turbidity of juices and concentrates are due, in large part, to these compounds. Furthermore, the conditions of juice preparation create opportunities for oxidative reactions involving polyphenols; thus the types of polyphenols present in finished juices are affected by juice-making procedures.

Different types of phenolic compounds react differently under juice making conditions. The catechin-type phenolics and the chlorogenic acid serve as good substrates for apple polyphenol oxidase, but the products from catechin oxidation are darker (Siegelman, 1955) and have a greater tendency to condense. The catechins oxidize more readily than the cinnamic acid derivatives. Flavonol glycosides are not direct substrates for the oxidase (Baruah and Swain, 1959; Walker, 1964b). It might be assumed that the amounts of particular phenolic compounds remaining in a juice would be as important as the total phenolic content in affecting the characteristics of the juice.

In recent years methods have been developed for the enzymatic treatment of apple pulp which improve both the ease of pressing and the yield of juice (Pilnik and de Vos, 1970; Verspuy et al., 1970; de Vos, 1970). The enzymatic treatment, employing pectolytic preparations, was most advantageous when used with apples of poor pressing characteristics, either due to variety, i.e. Golden Delicious, or to deterioration during storage. Since such apples are forming an ever greater part of the apples used for juice production the enzymatic procedure could be of considerable practical importance. Therefore it is desirable to have information on the polyphenol composition of juices derived from enyzme-treated pulp in order to provide a basis for the use of the process by the apple juice industry. Some of the effects of such treatments on the character of apple juice have already been described (Pilnik and de Vos, 1970; de Vos, 1970) and include changes in the phenolic content, the color, and the aroma.

Sprenger Instituut and Agricultural University, Wageningen, Holland.

[1] Permanent address: Department of Food Science and Technology, Cornell University, Geneva, New York 14456.

The major cinnamic acid derivative in apples, chlorogenic acid, has been measured in a number of varieties (Kuusi and Pajunen, 1971; Macheix, 1970; Walker, 1963) and amounts in apple flesh are reported as 20 mg to 200 mg per 100 g of fresh tissue. Little or no loss takes place during juice storage or concentration (Tanner and Rentschler, 1956).

Flavonol glycosides have been measured in apple epidermal tissues (Walker, 1964a; Workman, 1963) where they are concentrated. Typical values are between 0.5 and 18 mg per g of fresh peel. These are mainly glycosides of quercetin having galactose, glucose, arabinose, rhamnose, or xylose as the sugar group (Fisher, 1965; Siegelman, 1955).

While a number of the particular phenolic compounds of apples have been identified (Durkee and Poapst, 1965; Fisher, 1965; Van Buren, 1971; Van Buren et al., 1966) many of the studies on the phenolic content of apple juices have involved the determination of classes of compounds, such as total phenols, vanillin–sulfuric acid reactive phenols, and leucoanthocyanidins. None of these is a distinct or an exclusive group of phenolic compounds although studies of these classes have provided considerable insight into changes occurring in apple juice. In particular, the ratio of total phenolic groups to vanillin–sulfuric acid reactive groups has provided a measure of the degree of polymerization of the phenolic compounds indicating that oxidation in a juice or pulp results, initially, in an increase in the degree of polymerization of the phenolic material (Pilnik and de Vos, 1970) accompanied by a rapid loss of vanillin–sulfuric reactive material,