The Effect of *Penicillium roqueforti* on Some Lower Fatty Acids[1]

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THE ABILITY of *P. roqueforti* to hydrolyze fat has long been known. In Roquefort cheese the flavor development is due, in part, to the breaking down of the fat through the action of this mold. Several investigators have concluded from their studies on *P. roqueforti* that the mold has the power not only to hydrolyze butterfat (1) but also to use some of the resulting fatty acids as a source of carbon (2) (3). Because of the general action of *P. roqueforti* on butterfat, an attempt was made to determine the effect of the mold on some of the lower fatty acids.

In preliminary experiments plates were poured, using a 1.5 percent agar to which mold spores and various amounts of butyric acid were added. The amounts of acid used per plate were none, 0.0001 cc., 0.001 cc. and 0.01 cc. The plates were incubated several days at room temperature and then observed for mold growth. The plates containing no butyric acid showed no growth. Those containing 0.0001 cc. showed sparse growth, those containing 0.001 cc. showed good growth and spore formation, while those containing 0.01 cc. showed no growth. The plates supporting mold growth were free from any odor of butyric acid; while the uninoculated checks, containing the various amounts of butyric acid, had a pronounced odor. These results suggest that butyric acid, up to a certain concentration, made possible the growth of *P. roqueforti* and, since the odor of butyric acid vanished with the appearance of mold growth, it probably was being used as a food constituent.

The effect of *P. roqueforti* on butyric, caproic, caprylic and capric acids was further studied by adding these acids to skim milk, which had been coagulated by *Streptococcus lactis,* and then inoculating the milk

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with *P. roqueforti* spores. This medium was used in an attempt to approximate the conditions in Roquefort type cheese. The skim milk was placed in containers which gave a large surface area. In the first 3 trials 500 cc. of skim milk in 6-liter Erlenmeyer flasks were used, while in the remaining 6 trials 300 cc. of skim milk in 2-liter Erlenmeyer flasks were employed. The milk was sterilized in an autoclave and coagulated with a pure culture of *S. lactis.* The flasks were paired and each of the acids added to one pair. In the first 3 trials 0.1 percent of the acids was used. Except with butyric acid, this concentration proved toxic to the mold; therefore, after the third trial, the amounts of caproic, caprylic and capric acids were reduced to 0.05 percent. Mold spores were added to one flask in each pair and all flasks allowed to incubate at room temperature. The length of incubation varied with the rate of mold growth. After a thick felt of mold growth had formed and proteolysis of the curd had begun, the cultures with their control were slightly acidified with sulfuric acid and distilled with steam. The data obtained from these distillations are presented in table 1.

The most significant fact shown in the table is that the growth of *P. roqueforti* in every case caused an appreciable reduction in volatile acidity. This reduction was largest for butyric acid and gradually decreased as the molecular weights of the acids increased. The most complete reduction of the volatile acidity was found in trial 7, where 94.21 percent of the butyric acid was destroyed. The other extreme was found in trial 5, where only 27.68 percent of the capric acid disappeared. It must be kept in mind, however, that after the third trial the percentage of butyric acid used was double that of the other acids.

The amount of volatile acid produced in the control flasks was always low. This is to be expected since cultures of *S. lactis* never produce a high volatile acidity; however, even this small amount was markedly reduced by mold growth. The values for the control flasks were so consistently low that after the first three trials control flasks were not used.

The ability to reduce volatile acidity did not seem to vary with the different strains of *P. roqueforti* used. However, the appearance of the growth in the flasks suggested there was a difference in their ability to proteolyze the curd.

It was pointed out by Currie (1) that *P. roqueforti* hydrolyzes the fat in Roquefort cheese and that the resulting volatile acids are responsible, in a large part, for the flavor and peppery sensation so characteristic of Roquefort cheese. Thus, the typical flavor of a Roquefort type cheese does not appear until some time after the development of the mold. Since *P. roqueforti* was found to destroy certain of the lower fatty acids that were added to a medium on which the mold was grown, it might appear that the mold destroyed the flavor instead of producing it. However, there are other possibilities that should be considered. The fatty acids freed early in the ripening period may be used up by the mold and then later,

TABLE 1. *The effect of P. roqueforti on some lower fatty acids*

Cultures incubated at room temperature (about 21° C.).

Trial No.	Mold culture used	Acid added to milk culture of *S. lactis*: Kind	Acid added to milk culture of *S. lactis*: Percent[1]	Days of incubation after inoc. of mold	cc. N/10 acid in 1,000 cc. distillate from 250 gm. culture: Without mold growth	cc. N/10 acid in 1,000 cc. distillate from 250 gm. culture: With mold growth	Percent acid destroyed
1	No. 6	none	......	10	6.9	2.8	....
		butyric	0.1	10	33.4	3.7	88.93
		caproic	0.1	31	27.8	3.7	86.70
2	No. 6	none	......	10	6.0	2.1	....
		butyric	0.1	10	39.2	2.6	93.40
		caproic	0.1	31	25.3	3.2	87.36
3	No. 6	none	......	10	9.1	2.9	....
		caproic	0.1	31	35.1	2.8	92.03
4	No. 6	butyric	0.1	12	31.1	2.3	92.61
		caproic	0.05	17	18.1	2.5	86.19
		caprylic	0.05	17	15.8	3.6	77.22
		capric	0.05	12	13.5	6.2	54.08
5	No. 6	butyric	0.1	12	34.1	2.7	92.09
		caproic	0.05	12	17.4	3.7	78.74
		caprylic	0.05	12	14.6	4.5	69.18
		capric	0.05	12	11.2	8.1	27.68
6	No. 6	butyric	0.1	12	33.2	3.6	89.16
		caproic	0.05	16	18.2	3.3	81.87
		caprylic	0.05	16	15.4	5.2	66.24
		capric	0.05	12	9.9	6.2	37.38
7	No. 3	butyric	0.1	21	32.8	1.9	94.21
		caproic	0.05	21	15.7	2.3	85.31
		caprylic	0.05	21	12.8	3.6	71.88
		capric	0.05	21	9.5	3.2	65.27
8	No. 8	butyric	0.1	16	29.8	3.6	87.92
		caproic	0.05	16	18.8	3.5	81.39
		caprylic	0.05	16	14.3	4.2	70.63
		capric	0.05	16	11.4	6.4	43.86
9	No. 9	butyric	0.1	21	32.8	2.3	92.99
		caproic	0.05	21	15.7	3.7	76.44
		caprylic	0.05	21	12.8	3.7	71.10
		capric	0.05	21	9.5	4.7	50.53

[1] Percent by volume.

when mold growth has practically ceased, the fatty acids could accumulate as a result of the activity of the mold lipase.

In a skim milk medium, coagulated by *S. lactis,* to which various fatty acids had been added, *P. roqueforti* caused an appreciable destruction of the volatile fatty acids. This suggests that the mold used the volatile acids as food constituents.

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