Production of Blue Cheese Flavor *via* Submerged Fermentation by *Penicillium roqueforti*

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A submerged fermentation procedure for the production of Blue cheese flavor is described. The process utilizes a spore-rich *Penicillium roqueforti* inoculum, developed by culturing the mold in sterile milk-based medium with a relatively high osmotic pressure. The flavor is produced in a sterile milk-based medium to which enzymatically hydrolyzed milk fat is added in a programmed manner. The fermentation is conducted under pressure, with low aeration rates. Flavor production occurs in from 24 to 72 hr. When optimum flavor production has occurred, the product is heat treated to inactivate the mold. The resulting flavor contains 7 to 12 times the ketone content of good quality commercial Blue cheese, but exhibits a flavor efficacy of four times that of Blue cheese. The product is used commercially in salad dressings, snacks, and appetizers.

Mold ripened cheese dates back to the Roman era, when it was made in France. Mold ripened cheeses are presently produced in major dairying countries around the world. There are at least 10 distinct varieties wherein typical flavor characteristics result primarily from the action of a mole species, usually a *Penicillium*. The popularity of mold ripened cheeses is steadily increasing. The most popular variety is Blue cheese, produced from cows' milk and cultured with *Penicillium roqueforti*.

Concurrent with the increasing popularity of mold ripened cheese sold and consumed in natural form, there has been a growth and proliferation of food products flavored with, or in simulation of, mold ripened cheese.

Many of these products require sources of Blue cheese flavor which impart typical flavor characteristics, but which fulfill other requirements of concentrated, standardized flavor strength, low cost, flexibility of formulating, and stability.

This paper describes an approach to the production of Blue cheese flavor *via* submerged fermentation.

MOLD CULTURES UTILIZED FOR BLUE CHEESE FLAVOR PRODUCTION

Penicillium roqueforti is the principal species of mold utilized for flavor production in most mold ripened cheese, including Blue cheese. A number of *P. roqueforti* strains are used by cheesemakers in various parts of the world. A white mutant strain isolated by Knight *et al.* (1950) at the University of Wisconsin is used to produce a distinct variety of mold ripened cheese "Nu-world." The absence of pigmentation in the spores of this strain enable the production of a uniformly light-colored or fermented product.

Penicillium camemberti and *Penicillium caseisolum* are the cultures of choice in Camembert and some other mold ripened cheese varieties. The use of a *Mucor* and a *Rhizopus* species has also been reported by Kosikowski (1966).

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Lactic streptococci, particularly *Streptococcus lactis* are utilized in Blue cheese production, primarily for fermentation of the lactose to lactic acid. Anderson (1966) has reported that certain yeast cultures isolated from the surface of Blue cheese may play a role in Blue cheese flavor production.

OUTLINE OF BLUE CHEESE PRODUCTION

Blue cheese is traditionally produced from high quality pasturized whole cows' milk containing 3.5% milk fat and 9.0% solids not fat.

Penicillium mold culture may be added to the milk at the outset of manufacture or sprinkled on curd just prior to forming the finished cheese loaves.

Streptococcus lactis culture is added and the milk is clotted with a milk clotting enzyme such as rennet.

The subsequent curd treatments are controlled so that the curd granules knit together to form a loaf with relatively open texture. Loaves are usually punctured at the beginning of the curing process so that air can penetrate the interior of the cheese loaf. The cheese is salted to contain 2.5 to 3.0% sodium chloride.

Cool curing temperatures of 8 to 9° C, and high relative humidity conditions are maintained to favor luxuriant mold growth within and on the cheese loaves.

The composition of mold ripened cheeses specified in the Federal Standards of Identity is 42 to 46% moisture and not less than 50% fat in the dry matter (21 CFR, Sec. 19.565).

FACTORS WHICH ENHANCE FLAVOR DEVELOPMENT IN BLUE CHEESE

Freshly made Blue cheese exhibits little or no typical flavor development. From 60 to 90 days are required before the character and amount of flavor in natural cheese are considered typical and acceptable for marketing.

A number of approaches have been used to improve the rate and efficiency of Blue cheese flavor production within existing cheese technology. Hussong and Hammer (1935) discovered that the rate of flavor development in curing Blue cheese could

be greatly enhanced by initiating lipolysis of the milk fat at the beginning of the curing period. The initiation of lipolysis has become an established part of Blue cheese manufacturing technology. A portion of the cream is separated from the raw milk and homogenized, thereby inducing lipolysis by the native milk lipase.

The rate of flavor development has been stimulated by producing a granular curd product, rather than the traditional loaf. Kondrop and Hedrick (1963) describe a method in which granular Blue cheese is ripened at elevated temperatures. It is claimed that satisfactory body and flavor equivalent to fully ripened conventional cheese can be obtained in a ripening period of 10 days.

When natural Blue cheese is ground or comminuted, rapid flavor development ensues. However, the development of abnormal, undesirable flavors often occurs, and this procedure is thus unsuitable for routine, large-scale use.

The three methods just described for increasing the rate and amount of flavor development suggest that a suitable liquid fermentation method might provide a rapid, efficient method for Blue cheese flavor production.

DEVELOPMENT OF THE FERMENTATION PROCESS

The technical foundations upon which the submerged fermentation of Blue cheese flavor rest are primarily the research of Knight and his colleagues at the University of Wisconsin (Gehrig and Knight, 1958, 1963; Girolami and Knight, 1955; Knight, 1963). They conducted research on the conversion of milk fat to ketones by *Penicillium roqueforti* cultures. When an inoculum of vegetative mycelial fragments suspended in a nutrient growth medium was inoculated into a substrate containing intact milk fat, approximately 2 days elapsed before ketones were produced; whereas 5 to 6 days were required when the inoculum consisting of spores was used. The time required to produce ketones from an intact milk fat substrate was longer when a 7-day-old *P. roqueforti* culture was used than when a 2-day-old, substantially spore-free vegetative culture was used.

Initially, they concluded that the spores as such were relatively inert and that a vegetative inoculum, preferably a young vegetative inoculum, should be used. However, they also noted that the fermentation proceeded in two steps, an initial hydrolysis of milk fat, followed by a conversion of the resulting free fatty acids to ketones. They thus underscored the observation of Hussong and Hammer (1935) that the production of ketones could be hastened by the addition of fat splitting enzymes to the substrate.

Ketone production by *Penicillium roqueforti* in substrates containing added milk fat modified by lipase treatment was studied. Seven day old *P. roqueforti* culture produced ketones more rapidly than the young, substantially spore-free inoculum. Experiments using washed spore suspensions revealed that spores could convert free fatty acid to ketones in a matter of minutes under submerged aerobic conditions. A concentrated flavor could be obtained in a matter of hours instead of days.

Knight and his group thus established that young, active vegetative inoculum provides enzymes for hydrolyzing the milk fat, and then, as spores are formed by the inoculum, the free fatty acids are converted to ketones. The spores introduced a ketone group on the number 3 carbon atom and then decarboxylate a fatty acid as follows:

$$RCH_2CH_2CH_2COOH \xrightarrow{\text{spores}} RCH_2\overset{\overset{O}{\|}}{C}-CH_3$$

Research on the development of a commercial scale submerged fermentation process for the production of Blue cheese flavor was conducted by Watts and Nelson (1963). Essentially, the work can be divided into two major phases. First, techniques for production of suitable spore-rich inocula were evolved. Second, the fermentation process was developed. The fermentation process simulates to a considerable degree some of the major factors which result in maximum, typical flavor development in Blue cheese.

The mold cultures used for cheese production in the United States are most commonly grown on autoclaved bread cubes. Although this type of culture can be rather readily produced in large quantities, it was observed that significant contamination by other micro-organisms commonly occurred. No such contamination could be tolerated in the submerged fermentation process because abnormal flavor development resulted. Mold culture preparations for cheesemaking are also produced by harvesting spores from pure culture slants or flasks. The quantity of inoculum required for commercial scale fermentation made this approach impractical.

A procedure was developed for producing bulk mold cultures which encouraged maximum sporulation. The method is based upon the control of substrate composition and osmotic pressure. Autoclaved, homogenized milk is utilized as the basic substrate. Initially, 1 to 3% of salt and/or sugar is added *via* sterile solutions. This medium is inoculated with white mutant *Penicillium roqueforti* washed from pure culture agar slants. After initial incubation under aerobic conditions for 3 to 4 days, an additional 4 to 6% of salt and/or sugar is added and aerobic incubation is continued for an additional 1 to 2 days. Incubation temperatures can range from 21 to 25° C. The control of pH in this milk based substrate is not required, although the desirable pH is 4.5 to 5.5.

The fermentation process for the Blue cheese flavor also most commonly utilizes a medium based on sterilized, homogenized milk. However, blends of homogenized milk and reconstituted nonfat solids or whey solids provide suitable fermentation media. Sterile salt may be added to the media in amounts up to 7.0%, although overall flavor balance is more desirable at levels of approximately 3.0%. The use of a milk-based fermentation medium containing some salt parallels cheese technology.

The fermentation medium is inoculated with 1 to 3% of spore-rich culture. Free fatty acids, essential for rapid flavor development, are added *via* milk fat modified with the calf pregastric esterase described by Farnham (1957). The modified milk fat is prepared in advance by enzyme hydrolysis of a milk fat emulsion to a specified free fatty acid content, followed by heat inactivation of enzyme activity. Two to three sequential additions of the esterase treated milk fat mixture are made as the fermentation proceeds to simulate the progressive release of free fatty acids that occurs in Blue cheese manufacture *via* milk lipase action.

The aeration rate with filtered air is maintained at low levels, not exceeding 1 liter of air per liter of substrate per min. Higher aeration rates result in "stripping" and consequent loss of the volatile ketones as they are produced. Aeration rates are usually well under the 1 liter limit just quoted and the fermentation vessel is also pressurized at 15 to 25 psi. The low levels of aeration tend to parallel conditions within a loaf of Blue cheese, with the exception, of course, of pressure.

Flavor production during fermentation is monitored *via* determination of total ketone content by the method of Haidle and Knight (1960). After 24 to 72 hr of fermentation, ketone concentration is 7 to 15 times that found in Blue cheese.

Flavor quality is monitored organoleptically and can be monitored via glc.

When a specified level of total ketone production has occurred, the product is sterilized via ultrahigh temperature/short time at 130° C, for 4 sec. The sterilizing system is closed and pressurized and therefore no significant volatile flavor loss occurs. The sterilizing process tends to "smooth out" the flavor, thereby improving overall flavor quality.

CHARACTERISTICS OF THE FERMENTED PRODUCT

The final product is a light tan liquid resembling milk, with a composition approximating 4% fat, 5% protein, 8% carbohydrate, 5% salt, and 78% moisture.

The ketone profile is very similar to Blue cheese as judged organoleptically and as determined *via* gas-liquid chromatography. The opinion of the Food and Drug Administration is that the product is generally recognized as safe and can be described in ingredient listings as "Blue cheese flavor."

The ketone content of the product is standardized at approximately 10 times that found in good quality commercial Blue cheese. However, the flavor efficacy of the product is about four times that of Blue cheese. Thus, one part of Blue cheese flavor produced by fermentation is equivalent to four parts of Blue cheese. The difference between ketone concentration and flavor efficacy results primarily from the low levels of protein derived flavor profile in the fermented product. The fermented product does not exhibit the full "nut-like" background present in Blue cheese.

COMMERCIAL APPLICATIONS OF FERMENTED BLUE CHEESE FLAVOR

The product presently enjoys commercial applications in salad dressings, snacks, and party dips. Usually, the flavor produced *via* submerged fermentation is combined with natural or spray dried Blue cheese. The product is of particular value in formulas where dried Blue cheese is used since the relatively high ketone content with respect to flavor efficacy effectively counterbalances the Blue cheese volatiles lost in spray drying.

The fermented product also serves as a base for fortified flavors which combine the flavor profile produced *via* fermentation with synthetically compounded flavor profiles.

The primary disadvantage of the product is relatively poor retention of flavor in baked or open-cooked formulations. The liquid character of the product results in lower retention than is exhibited by natural Blue cheese.

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