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Biogenesis of Cheese Flavor

Thorvald Kristoffersen

Cheese flavor results from the action of microorganisms and enzymes on the carbohydrates, fat, and proteins of the milk and curd. A multitude of breakdown products contribute to the flavor, such as acids, particularly short-chain fatty acids and acetic and lactic acid, alcohols, aldehydes, ketones, esters, ammonia, amines, sulfides, and mercaptans. Characteristic flavor of cheese varieties is related to the concentrations and balance of relatively few key components. Control of flavor development is exercised through selection of microorganisms and enzymes, milk treatment, and manufacturing and curing procedures. However, ultimately the development of characteristic cheese flavor appears to be determined by the ability of protein-based sulfur groups to accept hydrogen resulting from oxidative ripening processes. Results leading to this conclusion are reviewed.

Over 800 different cheeses are recognized in the world and these possess a wide spectrum of flavors. Most cheese is produced from cow's milk but cheese is also made from the milk of other domesticated animals and from nonanimal sources, such as soybeans. The flavor of cheese is determined in part by the source of the raw material.

Collectively, over 100 volatile and nonvolatile potential flavor compounds have been identified in ripening cheese. With respect to their contribution to flavor, suppositions have centered primarily on acetic acid and other short-chain fatty acids, amino acids, alcohols, aldehydes, ketones, esters, ammonia, amines, sulfides, and mercaptans.

FLAVOR DEVELOPMENT AND BALANCE

Control of desirable cheese flavor most generally is presumed to begin with the manufacturing process during which the internal environmental conditions for subsequent biological activities are established. Agreement exists with regard to the essential role of acidification by bacterial action of the milk and curd. This involves the selection of proper types and strains of lactic acid bacteria and the formation of lactic acid at the proper rate and in the proper concentration according to the cheese variety. Agreement also exists that the degree of curd dehydration and the salt content affect cheese flavor development, and the addition of specific flavor-producing microorganisms or enzymes aids in the formation of characteristic flavor in such cheese varieties as Swiss, Blue, and Romano, but not necessarily in all varieties.

During the curing process, the external environmental conditions are controlled to allow for proper flavor development. These conditions vary widely according to the kind of cheese. Basically, cheese flavor results from the action of microorganisms and enzymes on the lactose, fat, and protein of the cheese. The breakdown of these components to lactic acid, fatty acids, and amino acids, respectively, has been termed the primary step of cheese ripening (Harper and Kristoffersen, 1956). With the exception of short-chain fatty acids, the primary ripening compounds contribute little to characteristic cheese flavor. Such flavor results from subsequent action of microorganisms and enzymes on the primary ripening compounds.

Attempts to relate the characteristic flavor and flavor quality of a given variety of cheese to a single or a group of closely related compounds have generally proved futile. More success has been achieved when the relative concentrations of products resulting from the breakdown of protein and fat (Harper and Long, 1956: Kristoffersen and Gould, 1960; Long and Harper, 1956) or protein and carbohydrate (Kristoffersen and Slatter, 1959) have been related to flavor. For example, for Cheddar cheese there appears to be a definite relationship between the relative concentrations of free fatty acids and hydrogen sulfide and flavor quality (Table I).

BIOLOGICAL FUNCTION OF SULFUR GROUPS IN CHEESE RIPENING

Despite the mass of knowledge which has been accumulated on cheese manufacturing and curing procedures, the microorganisms and enzymes involved during the ripening process, and the flavor compounds produced, many instances occur when cheese fails to develop uniform, full, high quality flavor, even under apparently ideal conditions. For the past several years our research has sought an explanation for this incongruity, with emphasis on the sulfur groups of milk and cheese protein. Evidence accumulated indicates that the sulfur groups of these proteins are intimately involved in determining the quality characteristics of cheese and that they exert control over cheese ripening in concert with other agents.

The state of sulfur groups in milk may be subject to time–temperature dependent storage changes as revealed by studies of H_2S from milk (Kristoffersen *et al.*, 1962). The amount of H_2S released by heat from milk stored at 37° varied with time of storage of the milk (Figure 1). Similar but delayed changes were shown for milk stored at 4 and 15°. Assuming that the amount of H_2S released from milk is a function of the state of the sulfur groups, and relatively high concentrations of H_2S are released from the -SH state and relatively low concentrations are

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Table I. Relationship of the Flavor Character of Commercial Cheddar Cheese to the Ratio of Free Fatty Acid and Hydrogen Sulfide Concentrations (Kristoffersen and Gould, 1960)

No. of lots	Flavor character	μequiv FFA/g: μM H_2S/100 g
5	Balanced	14.2
4	Sulfide	7.2
5	"Fermented"	27.9

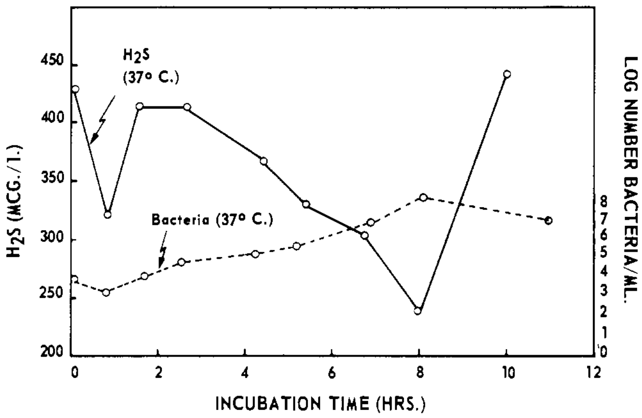

Figure 1. Changes in heat produced (90°, 30 min) hydrogen sulfide and bacterial numbers of fresh raw milk stored at 37° (Kristoffersen *et al.*, 1962).

Table II. Effect of Storage of Fresh Unheated Milk on the Concentrations of Active -SH Groups and Flavor of Cheddar Cheese (Kristoffersen *et al.*, 1964)

Storage time of milk at 37°	Active -SH groups, 2 months (mg cysteine HCl equiv/100 g of cheese)	Active -SH groups, 8 months (mg cysteine HCl equiv/100 g of cheese)	Flavor, 2 months	Flavor, 8 months
0	4.7	3.5	Very slight	Absent
5	5.5	13.0	Slight	Definite

Table III. Effect of Milk, Acidification, and Additives on the Concentrations of Active -SH Groups and Flavor Intensity of Cheese (Kristoffersen, 1967; Kristoffersen *et al.*, 1964; Singh and Kristoffersen, 1972)

Factor and type of cheese	Age of analysis	Active -SH groups	Desirable flavor
Season			
Swiss			
Winter milk	2 months	2.1	Very slight
Summer milk	2 months	6.7	Slight
Heat treatment of milk			
Cheddar cheese			
none	6 months	10.4	Definite
61.5°—5 min	6 months	7.0	Slight
61.5°—30 min	6 months	3.6	Very slight
68.5°—15 min	6 months	0	Absent
Acidification and minor constituents			
Cheddar slurry			
Lactic acid	8 days	1.3	Absent
Lactic acid, GSH	8 days	1.4	Absent
Lactic culture	8 days	1.6	Slight
Lactic culture, GSH	8 days	7.9	Definite
Lactic culture, GHS	8 days	10.6	Pronounced
Riboflavin, cobalt, diacetyl			

released from the -SS- state, then the results indicate that the sulfur groups of milk proteins are in a dynamic state.

The effect of the state of sulfur groups in milk on Cheddar cheese flavor was determined by manufacturing cheese from strictly fresh milk and a portion of the same milk stored at 37° for 5 hr (Kristoffersen *et al.*, 1964). Cheese manufactured from stored milk with the sulfur groups in the -SS- state developed fuller and superior flavor in comparison with cheese made from fresh milk with the sulfur groups in the -SH state (Table II). Furthermore, cheese made from the former kind of milk developed relatively high concentrations of active -SH groups during curing in comparison with that made of the latter kind.

The hypothesis is advanced that milk protein-based sulfur groups serve as a reservoir for hydrogen in biological oxidation-reduction processes during manufacture and ripening of cheese. At least a portion of the masked -SH groups of fresh milk must be oxidized prior to or during cheese manufacturing to produce -SS- groups which, in turn, must be available for reduction to active -SH groups during cheese ripening. The extent to which the oxidation of -SH groups progresses in the milk during storage and cheese manufacturing will determine the extent to which -SS- groups become available for deposition of hydrogen during cheese ripening, and the eventual development of -SH groups and flavor in cheese. When -SH groups are not oxidized and -SS- groups are not formed prior to cheese ripening, hydrogen resulting from oxidation-reduction reactions during the ripening process can not be deposited or is delivered elsewhere. In such cases different ripening products are produced, the balance of desirable flavor components is upset, and irregular cheese flavor results. Therefore, if this hypothesis is correct, the sulfur groups of milk and cheese proteins occupy an important role in the biogenesis of cheese flavor, and the control of desirable cheese flavor begins with the milk supply and not with the cheese manufacturing process.

Although the mechanism of hydrogen transport in milk and cheese has not been fully resolved, it would appear that well-established reductive microbial reactions are involved. Bacterial growth (Figure 1) and the formation of lactic acid (research in progress) may contribute to the transition of protein based -SH and -SS- in the milk during cheese manufacturing. During ripening, numerous reactions may be responsible for the -SS- to -SH transition. In Cheddar cheese decarboxylation of pyruvic acid to acetic acid and in Swiss cheese the formation of propionic, acetic, and carbonic acids from lactic acid may be major contributing reactions. Acetic acid has been stated to be the flavor compounds *sine qua non* in Cheddar cheese (Patton, 1963) and the onset of gas production coincides with the formation of -SH groups in Swiss cheese (Kristoffersen, 1967), both of these being consistent with the stated hypothesis.

FACTORS AFFECTING -SH GROUP FORMATION

The formation of active -SH groups in Cheddar and Swiss cheese has been related to a number of factors including: season of manufacture (Kristoffersen, 1967); heat treatment of milk (Kristoffersen *et al.*, 1964); acidification of the milk and curd with lactic acid bacteria (Singh and Kristoffersen, 1972); the type of culture and the rate and extent of acid development (Singh and Kristoffersen, 1971a,b); salt content of the cheese (Singh and Kristoffersen, 1970); and minor constituents, such as glutathione, riboflavin, cobalt, and diacetyl (Singh and Kristoffersen, 1970, 1972). Data in Table III show the contents of active -SH groups in cheese as affected by milk treatment, acidification, and additives. Figures 2 and 3 illustrate the overall changes in active -SH groups during cheese rip-

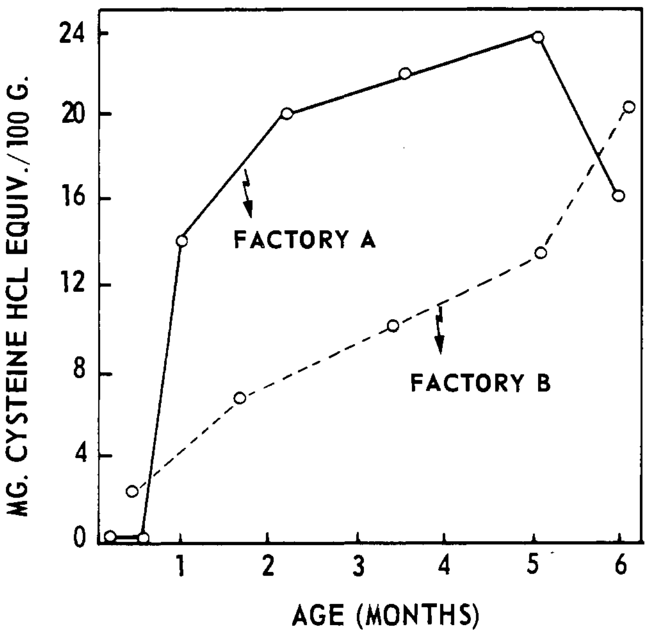

Figure 2. Changes in active sulfhydryl groups of Swiss cheese during aging (Kristoffersen, 1967). Reprinted with permission.

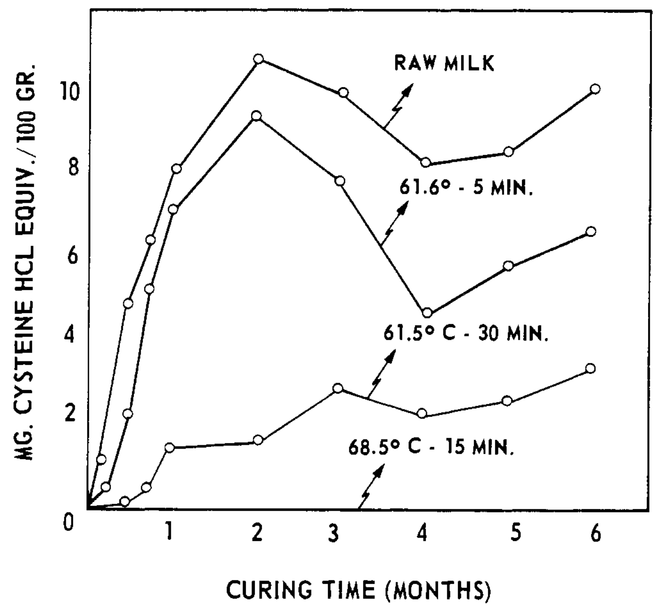

Figure 3. Changes in active sulfhydryl groups of Cheddar cheese manufactured from raw and heat-treated milk (Kristoffersen *et al.*, 1964). Reprinted with permission.

ening. In all cases, the formation of -SH groups has been related to the development of desirable cheese flavor.

With regard to the various factors, the effect of season, milk treatment, and acidification on active -SH groups and flavor formation in cheese may be on the extent of the transformation of -SH groups to -SS- groups in the milk and during curd manufacturing. The transformation would be retarded by low temperature storage of milk (winter); heat-induced interactions of -SH groups; and absence of or slow lactic cultures and low acid development. On the other hand, the effect of salt and minor constituents and cultures to some degree would be on the rate of transformation of -SS- to active -SH groups in the cheese during ripening.

With respect to cheese made from heat-treated milk, the lack of flavor of such cheese in comparison to that made from unheated milk is generally assumed to be the result of heat destruction of important bacteria. However, attempts to duplicate the flavor of cheese made from unheated milk by addition of various so-called flavor-producing bacteria to heated milk have been unsuccessful. The heat-induced interaction of sulfur groups of milk proteins, which reduces the ability of the groups to accept hydrogen during the cheese fermentation process (Table III, Figure 3), may be the real reason why flavor development in cheese made from heat-treated milk is less than in that made from unheated milk.

Our work on cheese flavor and -SH group formation has been aided greatly by the development of an accelerated process for curing of curd (Kristoffersen *et al.*, 1967; Singh and Kristoffersen, 1971b, 1972). By this process freshly made curd can be cured to a full-flavored product in 5–8 days. A significant finding has been that glutathione is an important factor in the formation of characteristic cheese flavor and active -SH groups (Table III), suggesting that it may serve a role as a mediator in hydrogen transfer.

It is increasingly apparent that before complete control of cheese ripening can become a reality, control of the milk supply in respect to the state of sulfur groups and the minor constituents affecting bacterial activities, particularly hydrogen transport, will need to be achieved. Some Swiss cheese manufacturers are practicing storage of the milk at slightly elevated temperatures prior to cheese-making with excellent results. Adoption of this practice has resulted in a large decrease in the percentage of undergrade cheese. However, the storage treatment is strictly on an arbitrary basis.

FLAVOR FUNCTION OF SULFUR

Sulfur groups have been discussed primarily in respect to their possible function in cheese ripening. Sulfur groups also contribute significantly to cheese flavor, and -SH and -SS- groups are readily detectable during the early stages of ripening of many varieties of cheese. The -SH and -SS- flavor becomes masked as other flavor components are formed, including sulfur-containing derivatives like sulfides and mercaptans. That sulfur groups are important flavor components of many cheese varieties is a point deserving of consideration in the formulation of synthetic cheese flavorings.

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