SYMPOSIUM: FLAVOR RESEARCH IN FATS AND FAT BEARING FOODS

Presented at the AOCS Meeting in Atlantic City, New Jersey

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Enzymatically Produced Flavors for Fatty Systems[1]

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ABSTRACT

Technology has been developed for the production of flavor systems via controlled enzyme modification of fats. Lipases and esterases from various sources are used. Fats modified include milk fat and meat fats. A wide range of flavor profiles can be produced via control of enzyme, fat and processing prior to and subsequent to enzyme modification. Applications for the flavor systems include margarine, imitation dairy products, confections and prepared foods.

INTRODUCTION

Enzymatically produced flavors are common to many types of foods, particularly those which are produced by fermentation. The primary emphasis of this presentation will be on one type of enzyme action—lipolysis or the enzyme-catalyzed hydrolytic cleavage of triglycerides. Of course lipolysis often occurs concurrently with other enzyme processes that result in flavor development. However to illuminate some of the salient factors in the production and application of free fatty acid flavor profiles, this discussion is limited to that technology in which lipolysis is the sole, or a dominant, enzyme reaction.

Spontaneous lipolysis has plagued many aspects of food production, probably since food production began to evolve. Most major classes of foodstuffs, including grains, meats and dairy products, can yield a variety of undesirable flavors resulting from lipolysis. The intensity and nature of many of these defects have earned for lipases a well deserved reputation for mischief. This reputation has been a principal obstacle for scientists and technologists who sought to control and utilize lipolysis as a useful and valuable part of food technology. But gradually during the past two decades, the technology and products of controlled lipolysis have gained widespread application in flavor technology.

EARLY DEVELOPMENTS

The reputation of spontaneous lipolysis among dairy technologists is, without doubt, no better than in other phases of the food industry. Dairy organoleptic scorecard systems penalize any hint of "rancid" flavors. However tradition, certain characteristics of milk and a few promising clues provided the basis for the first attempts to harness lipolysis to a useful end.

Lipolytic rancidity in farm-separated cream was a common occurrence. The butter churned from such cream often exhibited excessively coarse, pungent flavor for table use. However this type of butter was preferred by many cooks for preparing baked goods.

Other traditional dairy products exhibited some flavor characteristics that a few perceptive technologists sought to understand and exploit. Certain types of cheese, notably Italian and mold-ripened (Roquefort, blue), normally exhibit flavor notes that were associated, first organoleptically and then chemically, with free fatty acids, and hence presumably with lipolytic processes.

[1]One of 13 papers presented in the symposium "Flavor Research in Fats and Fat Bearing Foods," AOCS Meeting, Atlantic City, October 1971.

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TABLE I

Free Fatty Acid Profiles Released from Milk Fat by Several Lipolytic Enzymes

Fatty acid, C-	(1) Milk lipase, mol %	(2) Steapsin, mol %	(3) Pancreatic lipase, mol %	(4) Calf esterase, mol %	(5) Esterase pancreatin, mol %
4:0	13.9	(10.7)	(14.4)	(35.00)	(15.85)
6:0	2.13	2.89	2.05	2.52	3.60
8:0	1.82	1.52	1.39	1.31	3.03
10:0	2.99	3.74	3.26	3.14	5.48
12:0	2.74	4.00	3.82	5.08	4.35
14:0 Br[a]	0.78	0.55	0.24	0.83	0.84
14:0	7.67	10.70	10.10	13.16	8.53
15:0 Br	0.86	1.05	0.82	0.97	0.84
15:0	1.28	2.60	1.35	1.23	1.21
16:1 Br	1.73	1.50	1.22	2.01	1.68
16:0	21.60	21.60	23.99	15.93	19.30
17:0 Br	1.20	1.34	1.28	0.73	1.20
17:0	0.72	0.87	0.70	0.40	0.84
18:1 and 2	29.18	24.32	25.48	14.24	21.06
18:0	10.53	13.38	9.66	3.19	10.06

[a]Fatty acid fractions denoted "Br" are believed to be branched chain acids.

The earliest example of technology based upon lipolysis is the use of lipolyzed milk in chocolate. A prominent pioneer in chocolate processing stumbled upon a process for imparting unique flavor character to milk chocolate. Technical documentation of the process is not available, so the process remains a trade secret. The explanation which follows is based primarily upon technological deduction.

Apparently, to preserve adequate quantities of milk obtained in the June flush for use in the peak chocolate production during fall and winter, milk was condensed and sucrose added to produce sweetened condensed milk. Little was known about the time-temperature combinations required to inactivate milk lipase, particularly in the presence of sucrose which markedly increases the temperature required to destroy milk lipase. A large quantity of sweetened condensed milk processed early one summer was found to be extensively lipolyzed or rancid when sampled some months later. In desperation the lipolyzed product was added to milk chocolate. The resulting product exhibited a unique, appealing flavor which consumers enthusiastically accepted. To this day this milk process, in combination with other facets of the company's chocolate processing procedure, yields a chocolate flavor that is duplicated by no other producer.

In the early 30's, Hammer (9) and Lane and Hammer (12) developed a major innovation, based upon lipolysis, for the manufacture of blue cheese. Cream is separated from raw milk and homogenized. The homogenization activates the milk lipase. The homogenized cream is recombined with the skim milk and the mixture is made into cheese. The rapid lipolysis initiated by homogenization greatly accelerates the rate of typical blue cheese flavor development. About 25 years later, Knight and coworkers (6-8) elucidated the mechanisms in which short chain free fatty acids released from milk fat are the major precursors of the ketones which give blue cheese its characteristic flavor.

Hammer's contribution to blue cheese technology clearly demonstrated the usefulness of two attributes of milk: (a) the unique composition of milk fat, rich in short chain fatty acids; and (b) an abundant supply of lipase which can be readily activated by mechanical shear such as that produced by homogenization.

ENZYME SYSTEMS

Milk lipase and other nonspecific lipases, such as steapsin and pancreatin, attack milk fat in a nonspecific manner. That is, these enzymes release various free fatty acids in almost the same proportion that the acids are present in intact fat.

Table I includes the free fatty acid profiles released from milk fat by three broad-spectrum or nonspecific lipases; milk lipase (column 1), steapsin (column 2) and pancreatin (column 3).

Farnham and coworkers (4,5) discovered enzymes, secreted by oral tissues of certain young mammals, that differed markedly from broad-spectrum lipases, in that they specifically released short chain fatty acids from milk fat. Farnham's discovery grew out of a search for the source of the fat-splitting enzyme system present in the alimentary rennet pastes utilized in the manufacture of Italian cheeses. The typical flavor of these cheeses includes fatty acid notes. The enzyme system discovered by Farnham et al. is classified as a pregastric esterase. This classification is based upon the method of Desnuelle and Savary (2), who describe a method for differentiating lipases from esterases, based upon the enzyme activity on a substrate in solution form compared to the enzyme activity on a substrate in emulsion form. If the activity of a fat-splitting enzyme increases disproportionally on an emulsion form substrate, the

TABLE II

Activity of Various Enzymes in Triacetin Solution (5%) and Emulsion (15%) at pH 6.20

Enzyme	Activity, units/min: Solution (S)	Activity, units/min: Emulsion (E)	Ratio, E/S
Pregastric esterase	2.8	2.0	0.71
Pancreatic lipase	2.6	5.8	2.2
Fungal lipase	0.2	4.4	22.0
Milk lipase	4.6	2.2	0.48

TABLE III

Ratio of Activity of Various Enzymes upon 1-Monobutyrin, Dibutyrin and Tributyrin Substrates (Activity upon Tributyrin = 1.00)

Enzyme	Ratio of activity compared to tributyrin substrate (10% emulsion): Tributyrin	Dibutyrin	1-Monobutyrin
Pregastric esterase	1.00	0.04	0.00
Pancreatic lipase	1.00	0.74	0.95
Fungal lipase	1.00	0.26	0.15
Milk lipase	1.00	0.35	0.12

enzyme is classified as a lipase. Lipases are apparently more dependent upon absorption to the emulsion interface. Table II displays some data collected in our laboratory during studies of various fat-splitting enzymes under constant pH conditions (16).

The specificity of pregastric esterase for short chain fatty acids enabled a more adequate exploitation of the high short chain fatty acid content of milk fat. The flavorful short chain free fatty acids could be preferentially liberated. The free fatty acid profile released from milk fat by a calf source pregastric esterase is listed in Table I, column 4.

The concurrent or consecutive modification of milk fat by both short chain specific esterases and nonspecific lipases can produce free fatty acid profiles that are intermediate between the extremes produced by each type of enzyme. Table I, column 5, lists free fatty acid profile data for milk fat modified by a mixture of pregastric esterase and pancreatin.

LIPOLYZED MILK FAT PRODUCTS

Commercial lipolyzed milk fat products are produced in three principal forms. Most common are lipolyzed milk fat emulsions, which may vary in fat content from 25-95%; the usual fat content is 50%. Two powder forms are produced —a whole milk powder analogous in gross composition to unmodified whole milk powder, and spray-dried lipolyzed emulsion on a whey solids or whey solids-milk solids carrier.

A wide range of lipolyzed products are described in the patent literature. Otting (13,14) used steapsin to modify milk fat in milk. Kempf et al. (11) used milk lipase to prepare lipolyzed milk compositions, in which part of the volatile fatty acids released were removed by steam distillation. Farnham et al. (4,5) describes the use of "oral lipase" pregastric esterase for the preparation of modified whole milk powder.

Claus (1) describes a process for producing low moisture modified fats by using papain lipase. Pangier (15) describes the consecutive use of lactic cultures and lipolytic enzymes to produce modified milk fat products.

The essential manufacturing steps for lipolyzed products include: (a) preparation of condensed milk or butteroil substrate; (b) preparation of a standardized enzyme system in water; (c) combination of milk fat substrate composition and enzyme; (d) homogenization to form a stable emulsion, thereby promoting the maximum rate of enzyme activity; (e) incubation at a controlled temperature until a specified amount or degree of lipolysis has occurred; (f) pasteurization to completely inactivate enzyme activity; (g) final standardization, spray drying or formulating, and packaging.

We have attempted to blend purified fatty acids and diglycerides to synthesize flavor systems resulting from lipolysis of milk fat. The flavor of the fatty acid profile could be duplicated rather well. We were unable to overcome a pronounced coarse flavor which was characterized by most judges as reflecting poor integration of the numerous flavor notes present. Thus there is still much to be learned about the flavor chemistry of lipolyzed milk fats, particularly the role of the so-called nonvolatile constituents such as di- and monoglycerides.

However lipolyzed milk fat emulsions are very effective carriers for other flavor adjuncts. Synthetic fatty acids, diacetyl, butter esters, lactones and other flavor adjuncts are blended with the flavor profile released by lipolysis for a number of applications.

APPLICATION OF LIPOLYZED PRODUCTS

Major application groups for enzyme modified milk fat products include: (a) chocolate products, including milk chocolate, compound coatings and chocolate flavor syrups and beverages; (b) butter flavors, including margarine, butter creams and butter sauces; (c) milk and cream flavors, including coffee whiteners, imitation sour cream and imitation milks; (d) cheese flavor, particularly when the flavor profile of Italian-type cheese is desired.

Flavor profiles from lipolyzed milk fat can exert a considerable range of effect on food flavor. At very low addition levels, a sensation of richness is imparted without any detectable free fatty acid flavor character. As addition levels are increased, the flavors imparted resemble cream or butter. When addition levels are relatively high, the flavor imparted suggests cheese.

The surface active characteristics of both fatty acids and the di- and monoglycerides present in lipolyzed systems must be reckoned with. A tempering period of hours or even days is required to enable equilibrium to be established at the interfaces of various product phases—liquid, fat and solid. The lipolyzed flavor will appear to intensify as the equilibrium process proceeds. This intensification is sometimes mistaken for the presence of residual lipolytic activity.

LIPOLYSIS OF OTHER FATS AND OILS

We have researched the lipolysis of other types of natural fats. The most effective enzyme system for treatment of nonmilk fats combines esterase- and lipase-type enzymes. Table III, again from our work with constant pH systems (16), displays the relative activities of four different fat-splitting enzymes, pregastric esterase, pancreatic lipase, fungal lipase and milk lipase. Pregastric esterase has a relatively low activity on mono- and diglycerides, compared to other lipases. Pancreatic lipase has the highest relative activity on all three substrates tested—tributyrin, dibutyrin and 1-monobutyrin.

Edible grade, unrefined animal fats show considerable promise as sources of useful flavor profiles. Beef, chicken and pork fats, lipolyzed with mixed esterase-lipase systems, yield flavor profiles that have been evaluated in a variety of experimental applications.

Beef fat yields a flavor profile that is relatively low in soapy or bitter flavor notes, which exhibits a roast or cooked beef character. Lipolyzed chicken fat exhibits intense soapy character, but also exhibits an intense chicken-related flavor termed "grassy" by poultry flavor judges. Low levels of lipolyzed chicken fat are very effective in reducing or eliminating laking of chicken fat in chicken broth. Lipolyzed pork fat exhibits soapy flavor, but also an intense "piggy" flavor. Ordinarily, "piggy" flavor is shunned in pork products. However lipolyzed pork fat shows some promise for applications where lard must be replaced by other fats, without sacrificing the flavor contribution of lard.

We have prepared lipolyzed vegetable lipids such as soybean oil, cottonseed oil and coconut oil. The flavor profiles of these vegetable fats have thus far exhibited little promise of potential application. The profiles are intensely soapy or bitter.

Some years ago we also conducted a very limited study on the lipolysis of cinnamon and clove spice oils. There was some indication that a combined esterase-lipase system could modify and possibly intensify the flavors of these two spice oils.

POSSIBLE FUTURE TRENDS

We are well past the era when lipolysis or rancidity was considered only a plague and a nuisance. We may, however, have barely tapped the usefulness of lipolysis in flavor technology.

The most significant single factor in the development of useful lipolytic technology was the discovery and application of a variety of lipase systems, with differing specificities for fatty acid chain length, type of glyceride molecule and physical condition of substrate. The availability of short chain specific esterases and broad-spectrum lipases made it possible to produce a wide range of flavor profiles from milk fat. The range and possible combinations of various lipase systems have not yet been fully explored.

An analogous advance in lipolysis technology might well occur with the availability of a wider variety of fat substrates, particularly fats in which fatty acid composition is controlled, both in composition and structure. Then two major variables in enzyme technology, substrate composition and enzyme specificity could interact to multiply the potentially available flavor profiles. The possible substrate compositions, considering just fatty acids C_4 through C_{12}, are practically limitless.

Another potentially valuable area of lipolysis technology is suggested by the modification of unrefined edible animal fats and spice oils. Lipolysis might be used to release those flavor compounds which may be somehow bound within fat systems.

Flavor performance of lipolyzed milk fats, compared to synthetic mixtures of fatty acids and diglycerides, demonstrates that there is much to be learned about nonvolatile flavor constituents and their role in the chemistry of flavor compounds in foods and about the detection of flavor substances by the palate.

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[Received November 26, 1971]