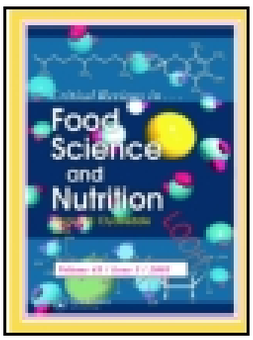

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FOOD ENZYMES

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INTRODUCTION

1. Food–Enzymes–Man

You are what you eat, according to an old saying. Disregarding the modifying facts, the saying brutally emphasizes the importance of food. Food makes man grow and enables him to exist and act. A sizable part of man's activity, in fact, centers around food. Gathering food, or raising plants and animals for foodstuffs and making them palatable, and working hard to earn the money to pay for this food are part of his major concern and the center of his thought. The excess or lack of food directs the actions of nations inside and outside of their borders and has been the instrument and goal of wealth, power, and wars.

Our body composition, what we are in flesh, is very similar to the composition of the food we eat. Present in both body and food are the substances commonly grouped as proteins, fats, carbohydrates, vitamins, enzymes, other related compounds, minerals, and water. These groups, representing thousands of compounds with definite molecules, with the exception of some minerals and the water, are produced by and are the parts of living creatures, plants and animals. They are manufactured by living cells through the activities of enzymes, themselves synthesized in the cell. In most instances substances in foods, constituting nutrients for man and synthesized by enzymes, are large complex molecules built from much smaller molecules. The complex molecules may exceed over a million molecular weight while the units of which they are built are around 200 or less. The human body depends on the small building units to build up its body substance or for releasing energy for its activities. Not the large but the small molecules can diffuse through the various membranes of the digestive tract, where the large molecules are digested and broken down to the small units.

Just as the synthesis of the constituents of the plant or animal cells is made by enzymes, their digestion, or breaking down, is done by enzymes in the digestive tract. These digestive enzymes are elaborated by the cells of the human body, or, if needed for ill persons, they are supplied orally. What you eat becomes you through decomposition and absorption and through recomposition and assimilation.

Milk, a complete food, a natural product formulated and manufactured by nature, is com-

posed of proteins, fat, carbohydrate, vitamins, and minerals in the best proportion for body building. And in addition to all of this, it even contains enzymes, including amylase, not needed for the digestion of milk. The milk amylase is a food additive provided by nature to digest starch if consumed with food other than milk.

Man, food, and enzymes are closely associated in life. This association created the food industry and a branch of it, the science of food enzymes. The primitive instinctive food appeal must satisfy four principal requirements: appearance, texture, odor, and taste. From a basket, man will select a peach with the prettiest color, soft but with a firm texture to the touch, and with the aroma only a ripe peach can emit. But if its taste does not meet expectations, he will throw it away and try another. Although mushrooms may smell and taste alike, the gourmand prefers the natural mushroom, because of its texture and appearance, to the mycelial or pelet mushroom propagated in fermenters in liquid media. Or, the color, odor, and taste of broiled chuck roast may be similar to chateau briand. Their food value is about the same. But chateau briand commands 200% higher price than does chuck roast just because of the difference in food appeal, mainly the texture.

The so-called appetizing food through its appearance, touch, fragrance, and initial taste stimulates the production of the digestive enzymes connected with the sensation of appetite. Enzymes in most cases play a vital role in developing food appeal. The science of food enzymes and the art of their application are based on this fact.[100]

Many flavor substances are formed from precursers through enzymatic actions, frequently within seconds after the tissues are cut or crushed. The flavor of chopped onion or crushed raspberries and of many other vegetables and fruits is formed that way, according to food scientists.[40]

When food enters the mouth in the first stage of the digestion process, it immediately contacts and is initimately intermixed with hydrolyzing enzymes present in the saliva.[11] In many instances, the initial dissimilation products are sensed by the taste buds which either approve or disapprove of the food. While a great many foods contain small molecular weight compounds instantly causing taste sensations in the mouth, many foods are composed of large molecules of nutrients with little or no taste. The utilization of many food enzymes is based on small-scale hydrolysis of the large food molecules, to produce just enough predigested hydrolytic substances to satisfy the flavor and taste demand.

The educated food appeal counts calories, selects balanced food components and, for special conditions, considers the digestability factors in preference to the natural instinctive food appeal. Again, to satisfy the educated food appeal, the food enzymes enter into action in the modifying preparation of natural foods.

Prevention of food spoilage is one of the principal objectives of food chemistry. Among the numerous compounds used as preservatives, enzymes, too, have found prominence.

2. Purpose–Objectives–Scope

The purpose of this review is to give a bird's-eye view of food enzymes–their sources, nature, utilization, trade names, grouping, and literature for the students of the various disciplines related to the subject as well as for the newcomers to food enzymology.

The objectives are to recognize and define the essentials and to refer to sources for details rather than to give exhaustive coverage and to point out and evaluate the issues instead of simply listing information collected from publications born out of diversified motives.

The scope only permits outlining the borders of food enzymes, their major characteristics, and their importance, merits, and problems. Full coverage has been brilliantly given within recent years by experts of the field[6,126] for those who wish to enter into intensive activities with any phase of food enzymes.

Only enzymes existing in food substances and added to them in the preparation of food will be considered. Microbiological processes connected with food preparation in spite of their enzymatic nature will be disregarded here, for they occupy a separate territory of food technology. The activities of yeast or other microorganisms in preparing bread, wine, beer, or other alcoholic beverages, the action of molds and bacteria in aging cheese, the production of vinegar fermented food products and similar process, therefore, will not be discussed. The use and action of commercial food enzymes in the preparation of beverages including beer and wine as they modify the taste appeal of the product in a manner similar to that of fruit juices or other food products fall within the scope of this review.

The literature survey presented here is also meant to serve as an introduction for more detailed studies. Sources, problems, trends, purposes, practicalities, and research and writing practices are the main avenues to be discussed and illustrated by specific examples in place of complete mapping of every alley.

One unique objective of this review, in conformity with the policy of this journal, is that it intends to be critical: critical principally with the orthodoxy of printed matter on food enzymes. By no means does the critic intend to pass judgment on publications or degrade their value, or lower the aims, techniques, or achievements of the research reported. The critic only wishes to illustrate the sluggishness, carried over from the past to the present, in serious efforts to streamline traditional practices.

The comments presented here will be those induced in the average reader by the writings. The purpose of recording them is to call the attention of future researchers and authors to evident shortcomings they should avoid when formulating and presenting their ideas, purposes, work, techniques, and results to the public.

In an effort to illustrate sources, trends, motives, and techniques of contemporary publications on food enzymes, it will not be possible to cover all the available literature. Only randomly selected items which may present an average cross section of the printed material will be reviewed. Many worthy publications will be omitted only for lack of space. Indulgence is requested of both the authors whose writings are reviewed and of those whose publications could not be included. It is hoped that this review will be helpful for those who wish to obtain an introductory knowledge in a short time.

3. Nature of Enzymes

a. Definition

The existence of enzymes was recognized and raw enzyme preparations were produced and named early in the last century. Kirchoff (1814) recognized that a substance germinating malt could saccharify the starchy paste; Payen and Persoz (1833) precipitated the active material with alcohol from an aqueous malt extract and named it diastase; Schwann (1936) discovered pepsin in the gastric juice.

As knowledge progressed, the definition of enzymes emphasized different characteristics at various times which ever seemed to be more distinctive at that time. Since Oswald, all definitions accepted the catalytic nature of enzymes, accelerating chemical reactions without being changed in the process.

Zemplen (1915)[152] underlined the colloidal nature of the enzymes produced by living cells; Waksman's (1924)[135] definition was, "An enzyme is a catalyst produced by living organisms and cells." Dixon-Webb (1958)[40] defined enzymes as: "A protein with catalytic properties due to its power of specific activation." This definition, for instance, excludes the non-protein active substances such as glutathione, cytochrome c, etc. from the group of enzymes. According to one of the popular definitions, "Enzymes are organic substances produced by living cells and have the ability of catalyzing specific chemical reactions."[32,33]

Recent reports[48, 64] on synthetic enzymes placed a dent in "enzymes produced by living cells." Still characteristic, the main limiting ideas—that enzymes are proteins, are colloids, are produced in nature by living cells, and that they catalyze specific chemical reactions—define the substances belonging to the group of enzymes.

Naturally, "food enzymes are enzymes active in preparation of food." The natural food substances contain considerable quantities of a great variety of enzymes which are active in turning the food substance into food by increasing its food appeal. The enzymes changing the banana, picked and transported green, into an appetizing ripe yellow fruit are considered food enzymes. Similarly, the proteolytic enzymes present in slaughtered meat, which under controlled conditions change the tough meat into a tenderized product, belong to the food enzymes. They modify the taste appeal of the original food substance.

b. Enzyme Characteristics[40,48,61,67,96,126]

Among the numerous characteristics of enzymes, the most essential are specificity, mode of action, kinetics, composition, their ability to be influenced by pH, temperature, activators, and inhibitors, by the enzyme substrate ratio, substrate, and concentration.

Most chemical catalysts accelerate a number of reactions. Not so with enzymes. Although a few enzymes may accelerate several very closely related reactions on very similar compounds, the majority of enzymes perform the catalysis of a

specific single reaction. The specificity may be selective enough to use certain purified enzymes to identify a substrate. The specificity is usually confined to the place and nature of the chemical bond where the enzyme acts.

c. Mode of Action

The typical enzyme reactions are illustrated by

$$E + S = ES = E + P$$

where

E	=	enzyme
S	=	substrate
ES	=	enzyme substrate compound
P	=	product

The enzyme molecule combines with the substrate molecule to form an enzyme substrate compound which quickly decomposes to free enzyme and the product or products. The enzyme, in quick succession, continues its task until the substrate is exhausted or an equilibrium is reached between substrate and product.

The science of enzyme kinetics and enzyme techniques, used for full characterization of any specific enzyme and of the mode of this action, is quite extensive and very elaborate and is well treated in comprehensive reviews.[24, 40, 56, 61, 69, 143, 144]

The International Union of Biochemistry[8] on the Nomenclature and Classification of Enzymes together with their Units and the Symbols of Enzyme Kinetics established certain standards for the symbols in enzyme kinetics:

$$(1)\quad E + S \underset{k-1}{\overset{k+1}{\rightleftharpoons}} ES \underset{k-2}{\overset{k+2}{\rightleftharpoons}} EP \underset{k-3}{\overset{k+3}{\rightleftharpoons}} E + P$$

k is used as a symbol of rate constant: capital K is used as a symbol of equilibrium constant.

(2) km is used for the substrate concentration at which v = v/2, known as the Michaelis constant. ks = the substrate constant used for the equilibrium (dissociation) constant of the reaction E + S = ES. ki is to be used for equilibrium (dissociation) constant of the reaction E + I = EI.

The velocity of any enzyme reaction under identical conditions depends on the enzyme substrate ratio. The velocity may vary from high speed to 0. Usually, in the initial phase with a high substrate to enzyme ratio, when the enzyme molecules are saturated with substrate, the reaction velocity is constant for a while and it depends on the concentration of the enzymes.

To understand the principles of the application of food enzymes and of their evaluation or measurement of their activity, it is sufficient to remember these simple facts.

d. Enzyme Composition[40, 94, 96, 126, 135, 152]

More than 100 individual enzymes have been purified in crystalline form, over 600 are produced in fairly purified form, and more than 1000 are provided with scientific names. The number of existing enzymes is estimated to be more than 10,000. Based on the study of crystalline enzymes they all possess a definite chemical composition and a molecular weight. The size of the molecules varies on a large scale, from 12,700 (ribonuclease) to one million or more (L-glutamate dehydrogenase, d-carboxylase).

They are all proteins, conjugated proteins, or metalloproteins, containing one or more active group in the molecule. The large molecules of conjugated proteins contain a relatively low molecular weight prostatic group, the coenzyme, usually in loose connection to the protein. The composition of many coenzymes is well known. They frequently contain some vitamin.

In the composition of the enzyme proteins, the number, type, and ratio of the aminoacids indicate no special difference from other non-enzyme proteins.

In studying the specific enzymes the following are considered: the molecular weight, the number of peptide chains, the sequence of aminoacids, the folding and arrangements of the peptide chains in the molecule, the number and nature of the active centers per molecule, the chemical nature of the active centers per molecule, the chemical nature of the active groups, and the structure of the prostatic group and its mode of attachment to the protein.

e. Factors Influencing the Enzyme Activity

The most valuable function of any specific enzyme, or, in practical terms, of an enzyme preparation, is its influence on the reaction speed. Reactions which proceed with infinitely low speed otherwise may be completed within seconds or minutes in the presence of sufficient amounts of the specific enzyme. Under proper conditions the reaction speed is doubled by using twice as much enzyme.

Since enzymes are expensive, the factors influencing their activity, or reaction speed, are essential. The major factors are pH, temperature, presence of activators, and presence of inhibitors. Enzymatic reactions usually are conducted in aqueous solutions or dispersions of the substrate although they may proceed in solids with low moisture content.

Each enzyme has an optimum pH at which its activity is the highest. The pH optimum may cover several 1/10 degrees on the scale but, occasionally, may be as wide as a full degree or more. The activity may drop sharply or gradually at pH levels below or above the optimum range. The slope is not even on both sides of the optimum range. For some enzymes the acidic is the more sensitive side and for others it is the alkaline. Frequently, a quarter of a pH change from the optimum range may reduce the activity one fifth to one third on the sensitive side and one third to one half on the less sensitive side. The pH optimum may be as low as 1.5 (for pepsin activity on egg albumin) and as high as 9.5 to 10 (for bacterial alkaline proteases).

The increase of temperature by 10^0C usually doubles the enzyme activity as long as the temperature does not permanently inactivate the enzyme. One mg enzyme preparation will catalyze the transformation of twice as much substrate at 30^0 C than at 20^0 C. The manufacturer or the distributor of food enzymes specifies the optimum pH and temperature of the product.

f. Inactivation

Enzymes may be inactivated temporarily or permanently. Lowering the temperature may deactivate the enzyme temporarily. As soon as the temperature is raised to optimum, the full activity returns. This is true to a certain limit. Some enzymes permanently lose part of their activity in aqueous solution if stored at very low temperature. Increasing the temperature above the optimum gradually destroys the enzymes. The thermoinactivation is usually permanent, and the enzyme is denaturalized and destroyed. The inactivation is the function of time and temperature. It is customary to determine the temperature which destroys 50% of the enzyme in a given (usually ten minutes) exposure time. The permanent thermal inactivation point is specific for each enzyme and varies on a large scale for the individual enzyme. Many food enzymes are considerably damaged at 45 to 50^0C. Malt amylases having an optimum temperature of 62^0C are destroyed at 75^0C within minutes.

Some enzymes are still active at extreme temperatures. Lipase enzyme of *P. roqueforti* slowly hydrolyzes milk fat at -29^0C. Six weeks' activity at this temperature equals the activity of 45 minutes at 37^0C. Several bacterial amylases and proteases are still active at 70 to 90^0C. The α-amylases of *B. stearothermophylus* retained 71% of its activity after 20 hours' exposure to 85^0 C.[96]

Chemicals such as strong acids, alkalis, oxidizing agents or strong proteolytic enzymes will permanently inactivate enzymes. Certain chemicals inhibit the activity of enzymes. Some inhibitions cause temporary (reversible) inhibition; others cause permanent inhibition (destruction). There are specific and general inhibitors. Irreversible inhibition is caused by poisons, cyanides, insecticides, and others. If temporary inhibitors are removed, by dialysis for instance, the enzyme activity is restored.

Regeneration

Some enzymes, milk catalase inactivated by heat for instance, when standing 24 hours are regenerated in part. Similarly, if bacterial amylase is inactivated by urea, after adding tribuffer to the mixture at pH 8.5, 80% of the enzyme activity will return.

Radiation by ultraviolet light or ionizing radiations as well as exposure to extreme high pressures (6000 atu) may cause permanent enzyme inactivation. For details consult ref. 96.

g. Activation

Enzyme activators are usually discussed in four groups: (1) coenzymes, (2) the prostatic groups, (3) metallic activators, and (4) reducing agents. Most prominent as hydrogen carriers are Coenzymes I, II, and III, glutathione, ascorbate, cytochromes, cytochrome oxidases, aminogroup carriers, phosphate carriers, acyl group carriers, K^+, NH_4^+, Ca^{++}, Mg^{++}, Mn^{++}, Zn^{++}, Co^1 and other ions. For details consult refs. 40 and 90.

Enzyme manufacturers usually list the activators and inactivators in the description of their products.

4. Nomenclature–Grouping–Classification [8,16,32,33,40,96]

Since nearly all types of industrial (commercially available in substantial quantities) enzymes

are applied or are applicable in some way as food enzymes, the general enzyme nomenclature, grouping, and classification are valid also for the food enzymes. Enzymes are described, named, and classified by various scientific groups such as enzymologists, biochemists, physiologists, cytologists, microbiologists, industrial manufacturers, biochemical and biological engineers, and food technologists.

In spite of the fact that they all tried to respect the historical, traditional, and commercially accepted names and groupings, they all made innovations and emphasized certain principles serving their particular fields. The result is a general trend with many small sidelines pointing in different directions.

The IUB[8] *Enzyme Nomenclature* is an excellent filing system for grouping and naming enzymes. It would, however, be entirely impractical and even impossible to discard the traditional and popular names. Processing personnel will never say "beta--D–Fructofuranoside fructohydrolase" in place of "invertase."

In addition to this, food technologists find it most convenient to discuss food enzymes grouped according to the industry they serve, such as cereal products, dairy products, meats, vegetables, fruits, fruit products, wines, beer, syrups, candy, cocoa, chocolate, coffee, flavors, shelf life extenders, and digestive aids.

The various attempts to standardize the terms, symbols, units, and groupings of enzymes produced the Enzyme Nomenclature, Recomendations 1964 of the International Union of Biochemistry.[8] The 219-page booklet is a valuable landmark, worthy to be considered by all students of enzymes. Since it only claims to be a recommendation–although its content was discussed and approved by the International Union of Biochemistry Commission of Editors of Biochemical Journals at its Meeting in Rome in February 1964– obviously it is not the final word in the efforts to unify the nomenclature and classification of enzymes.

The Recommendation formulated 31 rules to be followed for the systematic and trivial nomenclature. All enzymes are placed in six main groups:

1. Oxidoreductases
2. Transferases
3. Hydrolyses
4. Lyases
5. Isomerases
6. Ligases (synthetases)

Each individual enzyme is identified by a number. The first figure indicates to which of the six main groups the particular enzyme belongs. The second figure indicates the sub-class, the third figure indicates the sub-subclass, and the fourth figure is the serial number of the enzyme in its own sub-subclass.

Appendix E lists more than 1000 enzymes, giving their (1) number, (2) systematic name, (3) recommended trivial name, (4) other names, not recommended, (5) reaction, and (6) notes on specificity and other comments.

For example, the "Invertase" enzyme known to hydrolyze sucrose to glucose and fructose is listed as follows:

1 Number: 3.2.1.26, signifying that the enzyme belongs to the 3d group "Hydrolases;" 2 signifies the subgroup that acts on glycosyl compounds; 1 signifies that it belongs to sub-subgroup "Glucoside hydrolyses;" and 26 refers to its serial number in the sub-subclass. There are 39 enzymes listed in this sub-subclass; invertase is no. 26 in the line.

2 *Systematic* name is: α-D–Fructofuranoside fructohydrolase

3 *Recommended trivial* name is: β–Fructo–furanosidase

4 Other names (not recommended) are: sucrase, invertase, invertin, saccharase, β–h–fructosidase (the old traditional names)

5 Reaction: α–D–fructofuranoside $+H_2O$ = an alcohol +D–fructose

6 Notes on specificity and other comments: Substrates include sucrose; (it) also catalyzes fructotransferase reactions.

It is comforting to know that a system exists where among more than 1000 specific enzymes, "invertase," one of the oldest food enzymes, is listed and branded with the serial number 3.2.1.26, even if its old name is not recommended for use.

The Enzyme Nomenclature lists in 37 points a Summary of Recommendations: 6 on enzyme units, 6 on symbols of enzyme kinetics, 3 on the nomenclature of the nicotine-amide nucleotide coenzymes, 9 on classification and nomenclature of cytochromes, and 13 on classification and nomenclature of enzymes.

"Appendix A" lists 52 documents considered by the Enzyme Commission, many of them authored by M. Dixon. The standing Committee on Enzymes considered 36 documents, many of them authored by E. C. Webb.

The Enzyme Nomenclature considered many aspects of the field, including the controversy concerning the term "enzymic" versus "enzymatic." The commission preferred "enzymatic." Enzymic, being shorter, was mainly promoted by editors or journals and scientific books.

Dixon-Webb[40] lists 659 specific enzymes placed in four major groups, giving their (1) serial numbers (different from the IBU numbers), (2) active groups and cofactors, (3) names and sources, (4) substrates or reactions, and (5) references.

The book divides the 659 enzymes into three main groups: A. hydrolyzing enzymes, including 7 subgroups; B. transferring enzymes, including 8 subgroups; and C. other enzymes, including 4 subgroups.

No review can be given here on Dixon-Webb's *Enzymes*, but it is highly recommended for most details on enzyme studies, including the excellent mathematical and graphical treatment of Enzyme Kinetics, many useful charts, and 2353 references.

5. Occurrence in Nature

Enzymes, playing essential roles in every living cell, are produced and are present in every plant and animal cell. In higher organisms certain tissues produce and accumulate enzymes in much higher concentrations than others. Also, cells of specific tissues excel in producing one or several specific enzymes. In the animal world, glands engaged in supplying various digestive juices at different points of the digestive track are overwhelmingly active in producing hydrolyzing enzymes, most of them used as food enzymes.

Among higher plants, the seeds during germination usually excel in enzyme production. Several food enzyme preparations are produced utilizing germinating seeds. Some plant species produce and accumulate certain enzymes through nearly all their parts and others only in certain parts. Three important proteolytic food enzymes, papain, bromelin, and ficin, are made of plants. Papain is manufactured from the juice of the skin of unripened fruits of the papaya plant, *Carica papaya L.*; bromelin (bromelain), distributed throughout the pineapple plant, is produced from the juice pressed from the stems; and ficin is extracted from the latex of a tropical fig tree.

Microorganisms produce the greatest variety (hundreds) of enzymes in a single cell. Certain species are outstanding in producing a specific enzyme or several enzymes. The same species under different conditions may produce different enzymes in high concentrations. The amount of enzyme produced by such cells may exceed 100,000 times the amount of the same or similar enzymes produced by cells of other microorganisms for their own normal needs.

6. Manufacture of Enzymes[6, 13, 14, 17, 21, 25, 41, 63, 118, 122, 124, 129, 130]

On an industrial scale enzymes are produced in some or all of the following steps: (1) selecting the proper raw material, usually plant or animal tissues or microbial cells such as seeds, fruits, secretions, spleen, stomach, pancreas, stomach juice of young animals collected at packing houses, or cells of specific microorganisms; (2) enriching some of the special cells to produce enzymes, by germinating seeds, for example, in the manufacturing of barley or malt, or by propagating microorganisms under special conditions; (3) harvesting the enzymes from the cells usually by extraction; (4) concentrating the enzymes, either by eliminating the inert substances from the liquid or by precipitating the enzyme from the solution, for example by aceton; (5) stabilizing the enzymes to prevent loss of activity; (6) standardizing the enzyme potency to a desired level by adding inert substances to it or combining two or more enzymes for special purposes (formulation).

A special means of inducing cells to excessive enzyme production is to expose them to the plant hormone gibberellin. A few ppm added to the steep water will induce the production of about twice as much malt amylase in barley malt as would be produced without gibberellin.[27]

The enzyme production utilizing microbial cells includes the cell reproduction of the selected organism which, on final scale, may amount to multiplying a thousand times or more the number of cells used for inoculum. The propagation is usually conducted under aerobic conditions using a specially formulated liquid or solid medium and under controlled condition of the pH, temperature, and dissolved oxygen, and with the exclusion of foreign organisms.

Microbial enzymes may be endoenzymes re-

maining inside the cells or may be exoenzymes excreted by the cells into the medium. Accordingly, the harvesting of the enzymes is done by separating the cells from the medium and extracting them; or the enzyme may be harvested from the clear liquid medium after the cells are separated by filtration.

When used by the microbiologist, the terms "endoenzyme" and "exoenzyme" mean enzymes inside the cell wall or outside the cell wall. In the terminology of enzymologists "endoenzyme" means enzymes acting in the center part of a molecule; for example, alpha-amylase hydrolyzes the center parts of the gelatinized starch molecule. They use the term "exoenzyme" for the group of enzymes which act at the terminal ends of large molecules. For example, amyloglucosidase is an "exoenzyme," for it hydrolyzes the terminal ends of the starch molecule or its hydrolytic products with the formation of glucose molecules. These terms were first applied by the microbiologists. Recently, microbiologists resorted to the use of terms "intracellular" and "extracellular enzymes."

Microorganisms may produce toxic substances called "micotoxins;" best known of these is "aflatoxin" produced by *Aspergillus flavus.* All food and feed enzymes are tested for toxins before being approved and before being marketed.[36]

Production of industrial enzymes including food enzymes was reviewed.[33, 34] Most industrial enzymes are marketed in powder or granular form, but several are in liquid form.

II. FOOD ENZYMES [56, 69, 74, 75, 83, 96, 106, 107, 126]

1. Historical

Utilization of enzymes in preparing food obviously dates back before the age of written history. The early man who collected food must have realized that the stored food underwent changes within days—most frequently it was spoiled, but occasionally the changes improved the appearance, texture, odor, and taste. Unripe fruits in storage ripened and became sweet and flavorful; raw meat turned tender, tastier within days; hard seeds like cereals, beans, peas, and lintel when soaked in water became not only softer but also tastier and more palatable; on standing fruit juices and milk underwent changes often pleasing to the pallet.

In each case the changes were caused by enzymes already present or enriched in the stored fruits and soaked seeds and by microbial enzymes developed and acted in the juices or milk.

The art of making cheese, leavened bread, wine, and beer are ancient. Enzymes played essential roles in each process, first accidentally, then directed by practical experience as an art, and, finally, at the present time with full scientific explanations. The basic changes and their causes and results remained the same.

Today through accidental discoveries, carefully developed arts, and scientifically conducted experimentations a long line of enzymes is produced and marketed for improving the taste appeal of foods. Although they cover almost every possible field of food preparation, their number and application are still increasing. Table 1 lists the firms prominent in manufacturing and/or marketing food enzymes and enzyme sources in the U.S.

2. Commercial Food Enzymes

The primary purpose of all marketed food enzymes is to improve palatability, the food appeal of food ingredients and prepared food.[22, 45, 69, 78, 96, 114] In some instances, they may also improve the nutritional value; this, however, is hardly emphasized. Improvement of taste, odor, flavor, color, texture, and shelf life motivates the utilization, purchase, and manufacture of food enzymes. Some of them, like meat tenderizer and enzymes added to flours or cake mixes, are directly utilized in home cooking; more enzymes are supplied by restaurants. A great many are utilized by the prepared food industry such as dried food, frozen food, canned food manufacturing, fruit products, and wineries. Dairy products, bakery goods, pastry, cake, cookies, breakfast foods, cracker, confectioneries, and ice cream are made with the aid of enzymes; many food enzymes are used in the preparation of food ingredients, such as starch and dextrose syrups, dextrose, flours, and cake mixes and in the coffee, tea, and chocolate industries. The spreads, salad dressing, and other condiment manufacturers are users of food enzymes.

Pharmaceuticals, prepared for aiding the digestion of ingested food by persons deficient in their own digestive enzymes, do not serve the taste appeal. However, they do utilize food enzymes identical to those applied in preparing food; and they take part in the actual food digestion. They are, therefore, included among food enzymes

TABLE 1

Major Food Enzymes Marketed in the United States

Producer	Name and Source	Recommended Uses	Main Enzymes
Wallerstein Co. Staten Island, N.Y.	Fermex, *A. oryzae*	Bread, rolls	Amylase, protease
Wallerstein Co. Staten Island, N.Y.	Re-Tain, Bac. subtilis	Bakery goods, cakes	Alpha-amylase, heat stable
Wallerstein Co. Staten Island, N.Y.	Cellzyme, fungal	Reduce viscosity of plant mucilages; treatment of vegetables	Cellulase, beta -D-glycosidase, amylase, protease
Wallerstein Co. Staten Island, N.Y.	Enzyme 4511-3, Bact.	Add to flour used for chemically levened crackers	Protease, alpha-amylase
Wallerstein Co. Staten Island, N.Y.	Enzyme W, Bact.	For starchy syrups with low sugar count.	Alpha-amylase high temp. stable
Wallerstein Co. Staten Island, N.Y.	Amigase, fungal	Convert starch syrup to glucose	Amyloglucosidase
Wallerstein Co. Staten Island, N.Y.	Mylase, fungal	Convert hydrolyzed starch products	Alpha-amylase, limit dextrinase, other carbohydrases
Wallerstein Co. Staten Island, N.Y.	Malt Amylase PF barley malt	To hydrolyze low DE syrups to maltose	Alpha- and beta-amylases
Wallerstein Co. Staten Island, N.Y.	Tona	Meat tenderizer	Proteinases
Wallerstein Co. Staten Island, N.Y.	Proteases, fungal bacterial, vegetable	Meat tenderizer	Proteinases
Wallerstein Co. Staten Island, N.Y.	Klerzyme	To clarify fruit juices, wines	Pectinase
Wallerstein Co. Staten Island, N.Y.	Convertit, yeast	Sweetens and softens confections	Invertase (liquid)
Wallerstein Co. Staten Island, N.Y.	Dry Invertase, yeast	Manufacture of invert sugar, prevent crystallization in cane syrup	Invertase
Wallerstein Co. Staten Island, N.Y.	Papain, AS, C. papaya	Meat and fish tenderizer destruct urease in vegetable seeds, enhance the activity of malt diastase	Proteinase
Wallerstein Co. Staten Island, N.Y.	Prolase EB-21, bacterial	Modifies gluten and animal proteins, reduces viscosity of gelatin, produces little or no amino acids	Proteinase, no peptidase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Papain, C. papaya	Meat tenderizing, chillproofing beer, digesting tissues for rendering oils	Proteinase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Pancreatin-Takamine, pancreas	Digest aid	Amylase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Pancreatic lipase, pancreas	Digest aid	Lypase

TABLE 1 (continued)

Major Food Enzymes Marketed in the United States

Producer	Name and Source	Recommended Uses	Main Enzymes
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Bromelain, pineapple	Sugar wafers, waffles, pancakes, meat tenderizer, chillproofing beer	Proteinase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Spark-L	Hydrolyze pectins, in berry products, juices, grapes, apples	Pectinase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	DeeO	To stabilize egg solids, removes oxygen from bottles, canned or packaged foods extends shelf life	Glucose oxidase Catalase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Cellulase-Takamine	Clarification of citrus juice, brewing	Cellulase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Catalase B	Cold sterilization of milk for cheese, removing residual hydrogen peroxide from treated foods	Catalase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Fungal amylase, *A. oryzae*	Bakery goods, breakfast foods, chocolate and licorice syrups	Amylase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Diazyme	Manufacture of dextrose digest aid	Amylases incl. amyloglucusidase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Dextrinase A, fungal	Production of high (62-65) DE syrup	Amylases
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Clarase	In production of fruit juices, jelly, chocolate, baby food, clarifies liquids, chillproofing beer	Amylases, peptidase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Fungal Protease, *A. oryzae*	Production of soy sauce, mico (breakfast food in the Orient)	Proteinase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	Tenase	Production of corn syrup, baking, manufacture chocolate syrup	Alpha-amylase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	HT-1000	Liquefying for food	Amylase,proteinase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	HT concentrate	Converts starch in food processing	Alpha-amylase, proteinase
Miles Labs, Inc. Marschal Div., Elkhart, Ind.	HT proteolytic concentrate *B. subtilis*	Meat tenderizer, cereal breakfast foods, baking, crackers, cookies	Proteinase

Chas. Pfizer & Co., Inc. Milwaukee, Wisc.	Mashase	Preparation of brewery mash	Proteinases (papain, ficin)
Chas. Pfizer & Co., Inc. Milwaukee, Wisc.	Rennet, fourth stomach of suck-calves	Cottage cheese co-agulator, various cheeses	Proteinase
Chas. Pfizer & Co., Inc. Milwaukee, Wisc.	Metroclot (pepsin)	Cottage cheese co-agulator used in conjunction with rennet, manufacture of cheese	Proteinase
Chas. Pfizer & Co., Inc. Milwaukee, Wisc.	Tenderizing enzyme C. papaya	Meat tenderizers	Proteinase
Rohm & Haas Co. Philadelphia, Pa.	Pectinol 5-B, P. RA-5,P.10-M, P.59-L, P.R-10	Fruit juices, wine, jams, jellies, fruit concentrates, tomato, purees and pastes	Pectinase
Rohm & Haas Co. Philadelphia, Pa.	Pectinol 441-B concentrate	To reduce viscosity in above products	High polygalacturonase and low pectinesterase activity
Rohm & Haas Co. Philadelphia, Pa.	Pectinol 42-E concentrate	For the above products, for slow set pectin preparations	High pectinesterase and low polygalacturonase
Rohm & Haas Co. Philadelphia, Pa	Rhozyme A-4, R. J-25, *A.oryzae*	Bakery goods	Protease and amylase (ratio of the enzymes is different in the two products).
Rohm & Haas Co. Philadelphia, Pa.	Rhozyme 41, R. P-11 *A. Oryzae*	Meat tenderizing, removing meat from bones, bakery goods	Protease
Rohm & Haas Co. Philadelphia, Pa.	Rhozyme P-53, bact.	Processing meat and cheese products	Protease
Rohm & Haas Co. Philadelphia, Pa.	Rhozyme S, fungal	Converts gelatinized starch to dextrins, maltose, glucose	Amylases
Rohm & Haas Co. Philadelphia, Pa.	Rhozyme 33, fungal	Corn starch syrup	Alpha-amylase
Rohm & Haas Co. Philadelphia, Pa.	Rhozyme H-39, bact.	Dextrinise starch	Alpha-amylase active at 60-80° C
Rohm & Haas Co. Philadelphia, Pa.	Diastase D-7 concentrate	For dextrose and high dextrose syrups	Amyloglucosidase
Rohm & Haas Co. Philadelphia, Pa.	Rhozyme HP-150	To hydrolyze gums, mucilages, hexose and pentose polymeres, baked goods, chocolate syrups	Pentosanase, hexosanase, amylase
S.B. Penic & Co. New York, N.Y.	Papain *C. papaya*	Meat tenderizing, beer chillproofing, therapeutical use	Protease
Daryland Foods Labs Maukesha, Wisc.	Calalase lipase powder- K, kid glands	Promotes piquant flavor in cheese	Lipase
Dairyland Food Labs Inc. Maukesha, Wisc.	Italase lipase Powder C, calf oral glands	Produces special flavor in cheese	Lipase
Fermco Labs, Inc. Chicago, Ill.	Fermozyme CB-B	Removes sugar and oxygen, prevents	Glucose oxidase with traces of carbohydrases

TABLE 1 (continued)

		off flavor in drinks, mayonnaise	
Fermco Labs, Inc. Chicago, Ill.	Fermozyme 1307 liqu. microbial	Removes oxygen, prevents off flavor in orange drinks	Glucose oxidase low in trace enzymes
Fermco Labs, Inc. Chicago, Ill.	Fermozyme M liqu. microbial	Salad dressing, removes oxygen	Glucose oxidase
Wilson Labs, Inc. Chicago, Ill.	Pepsin, animal	Cheese manufacturing, brewing industry, instant cereals	Proteinase

Other major producers and suppliers of food enzymes are Armour Pharmaceutical Co. (Reheis Chem. Co.), Chicago, Ill.; Cudahy Packing Co., Omaha, Neb.; J. E. Siebel and Sons Co., Chicago, Ill.

Large food processing companies (Clinton Corn Processing Co., Division of Standard Brands, Clinton, Iowa, and Universal Foods Corp., Milwaukee, Wisc.) produce food enzymes for their own use.

Grain Processing Co., Muscatine, Iowa, and Midwest Solvent Co., Achison, Kans., produce technical grade amyloglucocidase; Enzyme Development Co. imports enzymes including food enzymes.

While the bulk of food enzymes takes part in the preparation (in most cases predigestion) of food substances, digest aids actually take part in the digestion of the ingested food.

The majority of food enzymes belong to the group of hydrolytic enzymes which, by the addition of hydrogen and hydroxy group taken from water, split the larger molecules into smaller units. They conveniently are grouped as (1) carbohydrases, hydrolyzing sugar polymers; (2) pectinases, hydrolyzing galacturonic acid ester polymers; (3) proteases, hydrolyzing amino acid polymers and related substances; and (4) lipases, hydrolyzing glycerids of carboxylic acid and other esters.

The non-hydrolyzing groups of food enzymes improve food ingredients by other reactions than hydrolysis. They fall into two groups: (5) oxidoreductases and (6) isomerases. Food enzymes, frequently containing traces of other enzymes than the main enzyme, may perform other intended or incidental changes. They may form an additional (7) group. Table 2 lists the principal food enzymes and their major fields of application.

Many enzymes identical to food enzymes (except for purity) are used to modify feed products. Since feed in most cases turns into food, it cannot be completely ignored by food technologists. In fact, many food ingredients with respect to potential presence of toxic substances (microtoxins, pesticides, fungicides, herbicides, gibberellin residues) and with respect to the presence of pathogenic organisms are under FDR regulations. Feed enzymes need the close consideration of food enzymologists.[78,125]

3. Individual Food Enzymes

Following is a brief account on the group of most common individual enzymes used as food enzymes.

a. Carbohydrases

Carbohydrases hydrolyze compound carbohydrates with the formulation of smaller molecules of compound carbohydrates and/or simple sugars. The substrates these enzymes act upon include large polysaccharide molecules such as starches, glucogens, inuline, cellulose, pentosans, and hemicellulose and their intermediate hydrolytic products such as dextrins, as well as oligosaccharides and trioses like raffiose, and simple disaccharides, such as sucrose, maltose, and lactose.

The individual carbohydrases are specific with regard to action site (linkage), characteristic temperature and pH optimum, and specific thermal tolerances.

Enzymes hydrolyzing starch are termed amylases.

α-Amylase– IUB 3.2.1.1, 2-1.4 Glucan 4- Glucano hydrolase, hydrolyzes 2–1, 4 glucan links in a random manner in polysaccharides containing three or more 2-1,4 linked D-glucose units, such as starch, glycogen, and related polysaccharides and oligosaccharides.

α-Amylase is present in saliva, pancreas, plants, molds, and bacteria. Frequently, it is called the "liquefying" enzyme because by random cleaving the large starch molecules, it reduces the gelatinized, thick starchy paste into a liquid. It is often used alone, as in the production of dextrins or starch syrups, but most frequently is used in

combination with saccharifying enzymes, as in the production of maltose, starch syrup, glucose syrup, and glucose. By cutting the starch molecules into smaller units, acting as an endoenzyme, it accelerates the action of the exo-enzymes, such as β-amylase and amyloglucosidase, which work at the ends of the chains. Alpha-amylase is active in maturing (curing) starchy vegetables.[38,45]

The cheapest and most common source of alpha-amylase is barley malt.[25] The food industry uses about three million dollars worth of malt, chiefly for α-amylase action. Alpha-amylase preparations are also produced from molds and bacteria. While malt α-amylase is quickly deactivated at 65° C, some microbial α-amylases are still active at 90° C.

β-amylase–IUB 3.2.1.2:α-1,4 Glucan maltohydrolase hydrolyzes the 2-1,4 glucan links in polysaccharides to remove successive maltose units from the non-reducing ends of the chains. It acts on starch, glycogen, and related polysaccharides and oligosaccharides, producing β-maltose by an inversion. β-amylase is widely distributed in nature. Malt is a rich source for both α- and β-amylases; certain animal glands and microorganisms produce considerable amounts of amylase.

Amyloglucosidase –(Glucoamylase or Glucamylase), IUB 3.2.1.3:α-1,4-Glucan Glucohydrolase hydrolyzes 2-1,4 glucan links in polysaccharides to remove successive glucose units from the non-reducing ends of the chains. It acts on starch, glycogen, and related polysaccharides and oligosaccharides.

Used for the production of glucose syrup and glucose, it is applied in conjunction with α-amylase. In addition, as a food enzyme it has been used in the conversion of distillery and brewery mashes. Many users of amyloglucosidase manufacture their own enzyme with the use of *Aspergillus* strains.[35,113]

Cellulase–IUB 3.2.1.4, β-1,4 Glucan glucanohydrolase, hydrolyzes β-1,4 glucan links in cellulose; principally a fungal enzyme, it also catalyzes transcellobiosylation.

Maltase–IUB 3.2.1.20, α-D-Glucoside glucohydrolase, or Glucosidase, hydrolyzes maltose into glucose and catalyzes glucotransferase reactions. It is present in many yeasts and other microorganisms.

Lactase–IUB 3.2.1.23, β-D-Galactoside galactohydrolase, or β-Galactosidase, hydrolyzes lactose and also catalyzes galacturotransferase reactions. The enzyme is present in *S. fragilis* and other microorganisms and in the digestive secretions of mammalians; it is utilized in ice cream manufacture, cakes and digestive aids, and has future potentialities.

Invertase–IUB 3.2.1.26, β-D-Fructofuranoside, or β-Fructofuranosidase, is also called saccharase. It hydrolyzes sucrose into invert sugar; it also catalyzes fructotransferase reactions. The enzyme is used in the production of invert sugar in the manufacture of candies, ice cream, cakes, and confectioneries. It is manufactured from *S. cerevisiae* and other microorganisms.[31,41,135]

b. Proteases

Proteins (albumins, globulins, gluteins, histones, protamins, scleroproteins, phosphoproteins and, conjugated proteins) with molecular weights from 16,000 to 17,000,000 are principally composed of a large number of aminoacids connected by the –CO–NH– peptide linkage. The number of aminoacids and their sequences, the proportion of the various aminoacids (about 20 different types), the inner connections, and the nature of the small amounts of non-aminoacid components in the molecule constitute the major difference among the various proteins. Since the living substance in the cells, in addition to the RNA and DNA, principally consists of proteins, they are the most essential and usually the most expensive group of food substances. The enzymes, themselves, are proteins. Proteins are hydrolyzed in the digestive track, and the small molecule simple hydrolytic products, principally the aminoacids, are absorbed and assimilated in the body.

Proteases, or proteolytic enzymes, acting upon the peptide linkage, catalyze the hydrolytic degradation of proteins. In an analogous way to endoamylases, there are endoproteases which act at random on the inner part of the protein molecule and on its hydrolytic products; and in an analogous way to the exoamylases, the exoproteases hydrolyze the terminal units of proteins and their hydrolytic products.

The science of proteases is somewhat behind the science of carbohydrases, not because of the lack of research but because of the more complicated structure of proteins in comparison to the simple structure of carbohydrates. In Enzyme Nomenclature[8] 24 proteases are listed under the subsubgroup 3.4.4 Peptidyl Peptide Hydrolases. This sub-subgroup includes most of the proteases used

TABLE 2

Principal Utilization of Food Enzymes

ENZYME	1 Meat, Fish, Vegetable, Microbial Proteins; Soy and Other Sauces	2 Milk, Dairy Products	3 Flour, Bread, Cakes, Cookies Crackers, Pancakes, Cereals	4 Syrups, Glucose, Ice Cream, Confectioneries	5 Eggs	6 Salad Dressings, Mayonnaise	7 Cocoa, Tea, Coffee, Chocolate	8 Fruit and Vegetable Products, Wine	9 Digest Aids	10 Shelf Life	11 Beer
A. Carbohydrates											
1. α-amylase			X	X			X		X		X
2. β-amylase			X	X							
3. amyloglucosidase			X	X							
4. other amylases			X								
5. invertase				X							
6. maltase											
7. lactase		X	X	X					X		
8. cellulase			X						X		
9. pentosanase			X						X		
B. Proteases							X		X		
10. papain	X										
11. bromelin	X										
12. ficin	X	X									
13. pepsin											
14. trypsin											
15. renin and microb. renin substitute		X									
16. bacterial and fungal protease	X		X				X				X
17. yeast protease	X										
C. Lipases											
18. glycerol-esterhydrolase		X	X				X		X		
19. gastric and other esterases		X					X				

	1 Meat, Fish, Vegetable, Microbial Proteins; Soy and Other Sauces	2 Milk, Dairy Products	3 Flour, Bread, Cakes, Cookies Crackers, Pancakes, Cereals	4 Syrups, Glucose, Ice Cream, Confectioneries	5 Eggs	6 Salad Dressings, Mayonnaise	7 Cocoa, Tea, Coffee, Chocolate	8 Fruit and Vegetable Products, Wine	9 Digest Aids	10 Shelf Life	11 Beer
D. Pectic enzymes											
20. pectin methylesterase							X	X			
21. polygalacturonase							X	X			
22. protopectinase								X			
23. pectic lyase								X			
E. Oxydoreductases										X	
24. glucose oxydase					X	X		X		X	
25. catalase		X					X				
26. peroxydase		X					X				
F. Miscellaneous											
27. glucose isomerase			X								
28. naringinase								X			

in food production. The International Union of Biochemistry assigned to these enzymes I.U.B. numbers but has not yet made any recommendations for their names. This is the only subsubgroup without names among the more than 1000 enzymes considered by the I.U.B. It is obvious that most commercially available food enzyme preparations contain more than one protease and that some of them may contain other enzymes than the 24 listed in this subgroup.

Following are the best known enzymes as principal agents in food protease preparations:

Pepsin–IUB 3.4.4.1 hydrolyzes peptides including those with bonds adjacent to aromatic or dicarboxilic L-aminoacid residues. Pepsin is formed from pepsinogen and is made from the glandular parts of the stomach of hogs. Pepsin acts at very low pH (1.3-3).

Rennin–IUB 3.4.4.3, also called chymosin, hydrolyzes peptides. Formed from prorennin, it is manufactured from the fourth stomach of young calves or lambs. It is used for coagulating milk, and it acts on the surface of the κ-casein molecule. Microbial rennin requires an acceptable name.

Trypsin–IUB 3.4.4.4, hydrolyzes peptides, amides, esters, etc. at bonds involving the carboxyl groups of L-arginine or L-lysine. Formed from trypsinogen, it is manufactured from pancreas.

Chymotrypsin A–IUB 3.4.4.5, and chymotrypsin B–IUB 3.4.4.6–are formed from chymotrypsinogens and are present in crude pancreatin preparations. They hydrolyze peptides, amides, esters, etc., especially bonds involving the carboxyl groups of aromatic L-aminoacids.

Pancreatopeptidase E–IUB 3.4.4.7, also called elastase, hydrolyzes peptides, especially at bonds adjacent to neutral amino acid residues. It is present in pancreas preparations.

Enteropeptidase–IUB 3.4.4.8, also called enterokinase, hydrolyzes peptides, converts trypsinogen into trypsin, originates from the intestinal epithelium, and it is present in the pancreatic juice.

Papain–IUB 3.4.4.10, and **chymopapain**, IUB 3.4.4.11, hydrolyze peptides, amides, esters, especially at bonds involving basic aminoacids, leucin, or glycine. They are produced from the excretion of unripe papaya fruits and have many applications in food preparation.

Ficin–IUB 3.4.4.12, hydrolyzes peptides, amides and esters; its specificity and application are similar to papain.

Subtilopeptidase A–IUB 3.4.4.16, also called subtilisin and produced by *B. subtilis*, hydrolyzes peptides and some simple aliphatic esters; it also converts ovalbumin into plakalbumin.

Aspergillopeptidase A–IUB 3.4.4.17, produced by certain *Aspergillus* species, hydrolyzes peptides, especially at bonds involving the carboxyl groups of arginine or leucine; it also converts trypsinogen into trypsin.

Bromelain–IUB 3.4.4.24, present in pineapple, hydrolyzes peptides, amides, and esters. Its specificity and use are somewhat similar to that of papain and ficin.

In addition to these proteases, many enzyme preparations contain enzymes (1) acting on ether bonds, IUB subgroup 3.3–thioether hydrolyses, (2) acting on peptide bonds, IUB subgroup 3.4-2-Aminoacyl Peptide Hydrolases, (3) acting on C-N bonds other than peptide bonds, IUB subgroup 3.5, hydrolyzing linear and cyclic amides.

Yeast carboxypeptidase–IUB 3.4.2.3 Peptidyl-glycine, hydrolyzes peptidyl-glycin to a peptide and glycin. Present in yeasts, it takes part in yeast hydrolysis among other proteases and other related enzymes. Yeast hydrolysates are essential food flavoring substances and ingredients of some health food products.[34]

c. Lipases

Lipase, or lypolytic enzyme, catalyzing the hydrolysis of fats and fatty acid esters, is placed by IUB in group 3, Hydrolases, in subgroup 3.1, enzymes acting on ester bonds, and in subsubgroup 3.1.13 named "Glycerol-ester hydrolase." Lipase catalyzes the hydrolytic breakdown of triglycerids through diglycerids and monoglycerids to glycerol and fatty acids. It is present in pancreas, and it is also elaborated by a number of microorganisms.

Lipases originating from kid, lamb, and calves' oral glands produce special flavors in cheese. Lipases are used to modify milk and chocolate products and are used in digestive aids.

Food enzymes contain a number of other esterases which may take part in modifying food substances and producing food flavors.

d. Pectic Enzymes

Taking part in the hydrolytic degradation of pectic substances pectic enzymes usually are discussed together for convenience, although they

belong to various groups of enzymes according to the reactions they catalyze (see IUB numbers). Pectic substances are discussed in detail by Kertesz[72, 73]; they were also reviewed very briefly by others.[32] The most important pectic enzymes are as follows:

Pectic mothylesterase or pectin esterase–IUB 3.1.1.11, pectin pectyl-hydrolase, hydrolyzing the ester bonds, transforms pectin into methanol and pectate (polygalacturonic acid). The enzyme is common in plants. The commercial products are manufactured from microorganisms.

Polygalacturanase–pectin depolymerase, pectinase, or pectolase, IUB 3.2.1.15, poly-α-1,4-galacturonide glycanohydrolase, hydrolyzes α-1, 4-D-galacturonide links in pectate and other polygalacturonides. By reducing the large molecular weight compounds into smaller units, eventually into galacturonic acid, it converts the pectic substance into water soluble compounds and reduces the viscosity of the solutions. It is extensively used in the production of fruit juices and wine.

Depolymerase–In hydrolyzing polygalacturonic acid, it requires the presence of pectin methylesterase.

Protepectinase–hydrolyzes protopectin, the substance that holds the joining cells together in plant tissues.

Pectate lyase, or pectate transeliminase, IUB 4.2.99.3, poly-2-1, 4-D galacturonide lyase. By eliminating Δ-4, 5-D-galacturonate residues from pectate, it brings about its depolymerization. It also acts on other polygalacturonides. Industrial food grade pectic enzymes may contain several of the pectic enzymes. Usually one is predominant, depending on the purpose for which the enzyme is intended.

4. Application of Food Enzymes

a. Enzymes in Cereal Products[18, 68, 81, 89, 96, 111, 112, 116, 127, 128, 137-141, 146]

The principal cereal products are bread and other bakery goods, breakfast foods, pancakes, doughnuts, crackers, cakes, and cookies. They are all made of grain flours composed mainly of starch, proteins, cellulose, pentosans, some sugar, fat, minerals, vitamins, and enzymes. The inborn enzymes of the flour are essential but not quite sufficient for improving the quality or taste appeal of the final food. A host of enzymes, including amylases, proteases, cellulases, pentosanases and lactase is recommended and used for this purpose. A great number of studies and reviews pointed out the biochemistry, technology, and quality improvement of the individual enzyme preparations usually containing one principal enzyme and several minor enzymes. The mode of action and the results caused by the same type of enzyme of various sources are somewhat different. These differences are frequently magnified by commercially oriented literature.

The most important enzyme actions are the modifications of the starch. One is the production of maltose and glucose for the yeast. Yeast ferments maltose, sucrose, invert sugar, and glucose but does not ferment dextrins or oligosaccharides containing more than two hexose units; some triozes are fermented slowly. The formation of carbon dioxide by yeast from sugar raises the dough and gives the volume and the spongy texture to the bread. Although sugar is added to some dough, the enzymatic formation of sugar through the action of alpha and beta amylases and amyloglucosidase is less expensive. The volatile components of the fermentation products, such as ethyl alcohol, traces of propyl-, butyl-, amyl-alcohols and isoamyl-alcohol, and aldehydes, acetoin, acetal, and other bouquet and flavor constituents are essential products in improving the taste appeal.

The ratio of sugar (enzyme produced or added) to yeast and the actual amount used in proportion to the flour are the main determining factors in the speed and processing methods in bread making. Special enzymes and enzyme mixtures are designed for mechanized and continuous bread making processes.[34]

Another factor is the enzymatic formation of dextrines and oligosaccharides, essential in the color and texture development of the crust of bread and hard rolls and in the formation of tasty substances. Dextrins, produced by alpha-amylase, play a part in the texture and aging of bread and in the toasting quality.

The inborn alpha-amylase content of healthy flours is generally very low; therefore, it must be supplied in the form of malt or fungal (*Aspergillus oryzae*) or bacterial (*B. subtilis*) amylase (BAA). Malt is the least expensive source, but it is destroyed at a lower temperature than bacterial alpha-amylases.

Flours contain beta-amylase, the enzyme that forms maltose from starch, and dextrin fragments produced by the alpha-amylase. Malt flour or malt

extract in addition to alpha-amylase supplies more beta-amylase to the dough.

Amyloglucosidase has been recommended and occasionally used in making bread. Amyloglucosidase or glucoamylase, an exoenzyme like beta-amylase, splits off glucose molecules from the ends of dextrin and oligosaccharide chains produced by alpha-amylase. While maltose must be hydrolyzed by the yeast before fermentation, glucose is readily fermented. Therefore, the utilization of amyloglucosidase adds more speed to dough raising when speed is a major consideration.

During sponge fermentation and floor and proof time, the amylases act upon the few percent of broken and fractured starch granules. In the oven they also have a few minutes' action time to attack the bulk of the gelatinized starch. The heat stable bacterial alpha-amylases act in the oven. On the other hand, if added in excess, they may over degrade the starch content, leading to crippled loaves and ropiness after a few days on the shelf.

Next in importance is the action of proteases on gluten, the main protein among the about eight different proteins in dough. Most dough also contains casein, added in the form of milk powder. The gluten must be conditioned by proteolytic actions to provide the best texture to the bread and rolls. A well developed stretchable gluten captures the carbon dioxide bubbles and the expanding alcohol fumes, thus giving the spongy texture to the bread. The gluten may be undertreated or overtreated by the proteases. The result of overtreatment is the flat bread.

Flours have inborn proteolytic enzymes; the yeast proteases take an essential part in gluten conditioning. Usually, fungal proteases are added to the dough for proper conditioning of the gluten. It is claimed that addition of proteases will (1) reduce dough mixing time, (2) help produce more mellow and drier doughs with good managability and handling qualities, (3) improve bread texture and enhance freshness, and (4) will result in a more uniform and symmetrical product with long shelf life. Similar claims are often made to justify the addition of other additives. Nevertheless, proteases are widely used in dough mixes by commercial bakeries; they would not apply them without positive advantage.

The oxidizing agents frequently added to the dough, by oxidizing the SH groups in gluten to S-S linkages, tend to tie the protein chains by a series of bridges. This will reduce the flexibility of the gluten, and "bucky dough" will result. Addition of proteases will produce sufficient peptides to reestablish the flexibility of the gluten.

In recent years, oxidizing enzyme systems in dough are gaining interest in view of some objection to the addition of oxidizing chemicals (bromates and iodates). Lipoxidases and invertase are also involved in conditioning the dough. BAA (bacterial alpha-amylase) is claimed to add, in addition to volume, proofing speed and sponge quality to the taste, aroma, texture, and shelf life of the bread.

U.S. Patent 3,026,205 (1962) claims that the use of alpha-amylase in 10 to 200 SKB units per 100 g of flour in the batter will reduce the normal crumb structure to a pudding-like mass before baking the batter. The alpha-amylase should be heat stable, preferably (second claim) produced by *B. mesentericus* and *B. subtilis*. The process may be applied to make chocolate, pecan, peach, coconut and other pies, nut and fruit baked confections, and sweet coated or iced crackers.

Among the advantages claimed for using the process are the following: time saving, assurance of success in home baking, delayed staling, a pudding-like structure, and a sugary pie filling taste. The process can be used to bake a sugary alpha-amylase treated layer on the surface of regular crackers.

Adding cellulase and/or pentosanase to the dough will increase the digestability, taste and texture, and color of rolls and bread.

Lactase enzyme, made by *Saccharomyces fragilis*, has been suggested as an additive to bread mixes containing milk powder. The lactose content, about one half of the milk powder, is not fermented by *S cerevisiae* (bakers' yeast), but it will become fermentable after being hydrolyzed to glucose and galactose by the lactase.

The browning of the crust of rye breads is connected with the actions of polyphenol oxidase.

b. Enzymes in the Production of Syrups and Glucose[19,35,55,59,71,96,99,103,131,145]

Carbohydrates, formed in more abundance than any other compounds, are the principal constituents of most agricultural crops raised for food or feed. Most prominent in human nutrition are the hexoses, principally glucose and fructose, and their condensation products, sucrose, maltose, and starch. Starch has somewhat higher food value, pound per pound, than the sugars, but it has little

or no taste. Therefore, its food appeal is way below the sweet tasting sugars. While sugars are consumed directly or are used to raise the food appeal of other food components, starch and starch containing cereals, as shown in the previous paragraphs, are generally modified to become palatable food products. Because of the great demand for sweet tasting sugar and because the price of can sugar was several times higher than that of starch, beginning early in the last century the industrial production of glucose from starch was successfully accomplished. The product obtained by the acidic hydrolysis of starch was not pure dextrose, but it was sweet and satisfied many demands where a low cost sweetener could satisfy the need. Called potato sugar in Europe and corn sugar in the U.S., the raw acid hydrolyzed starch product contained about 22% dextrose, 20% maltose, 20% tri- and tetrasaccharides, and 38% dextrins. Its dextrose equivalent was 42%.

Dextrose equivalent (DE) is the total amount of reducing sugars expressed as percent dextrose of the total dry substance. The manufacturing of high DE starch hydrolysates by acid conversion was limited by the unpleasant taste substances formed at higher DE levels.

In the early 1940's the acid-converted starch syrup (corn syrup) was further hydrolyzed by fungal amylase to 55 to 75 DE, most frequently to about 63 DE. The product was still high in maltose, oligosaccharides, and dextrins. The process was referred to as acid-enzyme duo conversion process.

The present methods utilize complete enzyme conversion for the production of corn syrup or glucose. A 27 to 33% corn starch slurry with pH 5.5-7.0 is heated to gelatinization temperature in the presence of a thermoresistant liquefying enzyme, bacterial alpha-amylase preparation, for about 30 to 40 minutes. The recommended amount of enzyme preparation (HT-1000) is 0.05 lb per 100 lb starch. The liquefied starch slurry is then cooled to 60°C, the pH is set to 3.5-5.0, and amyloglucosidase preparation (about 50 g per 100 lb of starch) is added. The mixture is then incubated for 72 to 96 hours at 60°C. Up to 97.5 DE values are obtained. One hundred pound starch on production scale may yield up to 105 lb dextrose.

Usually, less enzyme preparations and shorter conversion times are applied for producing corn syrups. Depending on the purpose for which the syrup is intended, the conversion may be interrupted at any desired DE value. For producing dextrose the conversion is carried out to the maximum level, the syrup is concentrated, decolorized, filtered, and the dextrose is crystallized.

Traces of other enzymes such as "transglucosidase," frequently present in alpha-amylase and glucoamylase preparations, lower the production of glucose due to the formation of oligosaccharides from glucose.

Several methods were developed for the elimination of "transglucosidase" from the amylase preparations. Glucoamylase preparations obtained by the use of *Aspergillus strains* are claimed to be free from or low in transglucosidase. The sweetness of glucose is somewhat inferior to sucrose; but because of its relatively low price, glucose finds numerous uses in the production of foods and soft drinks.

c. Enzymes in Meat Products

Meat tenderization, principally of beef, is generally practiced by storing the carcasses. In this (aging) process the natural proteolytic enzymes of the meat act on the proteins of muscle fiber. At 4°C the meat becomes tender in one to four weeks. At 15°C the tenderization is complete in three days. At this and at higher (30 to 40° C) aging temperatures, to prevent microbial growth and action the carcasses are exposed to ultraviolet light, during the aging period. The changes in the protein are connected with the formation of soluble non-protein nitrogen compounds.

Meat tenderization was practiced for centuries in the Western Hemisphere and the South Sea Islands, by using papaya leaves and fruit peels. The scientific and technological investigations date back to the 1940's. Meat tenderizers, proteases mixed with flavoring materials which are mostly monosodium glutamate, are very popular in outdoor grilling; much is used by frozen meat packers.[80,84,87,110]

Principally, papain ficin bromelain and fungal or bacterial proteases are used as meat tenderizers. In the U.S. about one third of the 600,000 lb of papain import per year is used for home meat tenderizer preparations. The enzymes work on protein, collagen, elastin, and mucoprotein components of meat. Tenderizers will not, or hardly,

affect the taste, flavor, or juiciness of the meat but will significantly improve the chewability.

Tenderizers are applied (1) on the surface of the cuts, after being forked, mostly in homes and restaurants using powdered preparations, (2) by dipping the cuts into dilute enzyme solution for a few seconds as practiced in restaurants and by frozen cut packers, (3) by injecting an enzyme solution containing a few ppm (5-30) commercial papain or other proteolytic enzyme into the beef five to ten minutes before slaughtering in the "ante-mortem" application, and (4) in the "post-mortem application" where the enzyme solution is injected at several places into the carcass after slaughtering but before the rigor mortis sets in. The uniform treatment of a whole carcass is somewhat difficult.

Poultry, especially old roosters and Tom turkeys, are tenderized by intravenous injection of 100ppm trypsin or 22ppm papain five minutes before killing the birds. The results are very satisfactory. Hams are best treated by adding a heat sensitive protease combined with phosphate salt into the pickling solution. When heating the ham to 66 to 68°C the enzyme is destroyed. If heat resistant enzymes survive the heating, the result will be a mashy over-tenderized ham.

Proteolytic enzymes are also used in the preparation of hydrolyzed protein solutions and soluble pastes as food or feed supplements. Fish solubles, a by-product of fish oil and fish meal production, are hydrolyzed by bacterial protease, clarified in a centrifugal separator, and the clarified liquid condensed into a soluble syrup. Without enzymatic hydrolysis the product would turn into a viscous, hard-to-dissolve mass. Fish solubles, a rich source of growth factors, are used in infant foods as well as in animal nutrition.

Render's meat scap, a by-product containing fat, meat, and bones, is ground and treated with proteolytic enzymes. The partial hydrolysis will loosen the protein particles from the bones. The meat portion is then separated in settling centrifuges from the bones and the liquid fat. Papain, bromelain, and fungal proteases are used in this process.

Oriental sauces, "fish sauce," and "soy sauce" are protein hydrolysates. Originally, they were made by natural fermentation processes, today by the use of proteolytic enzymes.

Yeast autolysate, a soluble protein rich paste, is made by the digestion of yeast cells by their own proteolytic and other hydrolyzing enzymes. The autolysis is induced by chemicals (acetone, lactic acid, etc.) by elevated temperature (45 to 55°C), or by added proteolytic and other lytic enzymes.[2,34]

Proteolytic enzymes are widely applied in the clarification of liquids containing protein precipitates or colloidal particles making the liquid cloudy or opaque especially when the liquid is chilled. Such liquids are beer, fruit juices, grape juice, etc. The cloudiness in beer, called "chill haze," caused by small amounts of undissolved proteins, may be eliminated by the use of bacterial (*B. subtilis*) and fungal (*Aspergillus, Mucor, Penicillium*) proteases. In addition to proteins, chill haze may contain some carbohydrates and tannin. Enzyme preparations recommended for prevention of chill haze usually contain several secondary enzymes in addition to protease to solubilize the haze substances other than proteins.

d. Enzymes for Fruit Products, Juices, Wines[23,26,72,73,134]

The colloidal material, mostly pectin, present in freshly pressed fruit juices, keeps dispersed solids in suspension. High viscosity and the dispersion of the suspended solids in tomato and citrus juices are essential; in such cases the pectic enzymes are destroyed by pasteurization to prevent the hydrolysis of the pectins. On the other hand, in many fruit juices, such as apple, cranberry, and grape, a high clarity and low viscosity are preferred. In the preparation of such juices a balanced mixture of pectic enzymes in the form of a commercial preparation is added, usually during the crushing. The breakdown of pectins will result in a free flowing juice where the solids settle quickly. Hydrolysis of the pectins in the crushed fruit also will increase the yield. Pectin treated juices can easily be filtered; and when pasteurized they are free from the boiled taste which is frequent in untreated juices after pasteurization.

Jellies and fruit vinegars made of pectic-enzyme-treated juices are superior in aroma, color, and brilliance. After pectin treatment prune juices may be concentrated to high solid contents.

For diabetics low sugar jellies are produced by using pectin esterase treated juices. By adding calcium, the low-ester pectin gels.

For the production of jellies the manufacturers

use pectic enzyme-treated highly clarified juices. The jellying is reestablished by adding commercial pectins, acids, and sugar.

Pectic enzymes are applied in different stages of wine making. If the enzyme is added to the crushed grapes, the advantages are reduced pressing time, increased yield, and an increase in the amount of free flowing juice. If the crushed grapes are fermented on the skins, the addition of pectic enzymes will increase the color of the wine. If the enzyme preparation is added to the must before or during the fermentation, the suspended particles, including unwanted microorganisms, will settle faster, the lee (yeast sediment) will be firmer and easier to remove, and at the first filling the wine will be clearer. Pectic enzymes may be added to the finished wine to produce a clearer wine and to reduce the filtration time.

It is claimed that pectic enzyme-treated wines are not only clearer and possess a better color, but they are also superior in flavor and bouquet. Flavors are frequently formed from precursers at the action of various enzymes.

e. Enzymes Used in Dairy Industry

Fresh milk contains considerable amounts of peroxidase, heat resistant alpha-amylase, and p-diamin oxidase; it also contains some lipase, alkaline phosphatase, acid phosphatase, xanthine oxidase, and catalase.

Pasteurization will destroy the lipase and alkaline phosphatase and will considerably reduce the amount of the other enzymes. By measuring the enzyme content, the degree of pasteurization may be estimated. The lipase present in milk plays a role in the ripening of cheese.

A number of enzyme preparations are used in treating milk and in the production of milk products.

The hydrogen peroxide–catalase treatment– The count of coliform and pathogenic bacteria is highly reduced by the treatment without destroying the natural enzymes and the lactic acid bacteria, most of which are destroyed by pasteurization. Enzymes and lactic bacteria are beneficial in cheese production. Hydrogen peroxide-catalase treatment is used where pasteurization is not feasible and in milk to be used for cheese.

There are two processes: (1) the flash and (2) the batch methods. In the flash method, about 0.01% hydrogen peroxide (100% basis) is added to the milk. The milk in a continuous flow is heated to 50 to 52° C for 25 seconds; then it is cooled and catalase enzyme is added to decompose the unused H_2O_2. In the batch method, about 0.02% H_2O_2 (100% basis) is added, usually at 30°C, and after 20 minutes' action time the excess hydrogen peroxide is decomposed by the addition of catalase enzyme. Completion of the decomposition of H_2O_2 is tested before using the milk.

Catalase is produced from the liver of slaughtered animals.

Rennet–The crude form of rennin called rennet, extracted from the stomach of suckling calves or lambs and kid goats, is used in curdling milk for making cottage cheese and a great variety of other cheeses. The enzyme in various crude forms has been used for thousands of years. The common practice of curdling or coagulating milk (the precipitation and floculation of the bulk of milk casein) in chemical terms is a rather complicated process. The casein particles in fresh milk are kept in suspension by a soluble casein fraction. Rennin acts on the surface of kappa casein (the calcium salt of which is soluble), and it liberates a few peptides. The equilibrium is disturbed, and the bulk of the casein floculates. In this oversimplified scheme the important fact is that, in reality, a very minute enzymatic modification on the kappa casein brings forth a dramatic change in the consistency of the milk. Calcium and phosphate ions take an essential part in the changes.

Similar change is produced in milk by most proteolytic enzymes, but rennin and pepsin are effecting the most complete curdling. Pure rennin is better than ten times more powerful than pepsin, and rennin produces the firmest curdling. The curdling occurs after a definite amount of enzyme action is completed. By measuring the curdling time, rennin and other proteolytic enzymes can be assayed.

In addition to rennin and, occasionally, pepsin, ficin and other plant proteases are also used in curdling milk, especially in countries where enzymes of animal origin cannot be used because of religious considerations.

The curds separated from the whey are used for making cottage cheese and other cheeses. Because the increasing demand for rennin coincides with the decrease of its natural resources, its price has more than doubled within recent years.[44] Many proteases were studied for replacement. The unique behavior of rennin is that after a quick and effective clotting of the milk by a limited pro-

teolysis, it has no further or very little action on the curd, while most proteolytic enzymes continue their hydrolytic activities with the result of dissolving the curds.

A number of microbial proteases were recommended for replacement of rennet. Among them was a promising enzyme produced by *B. cereus*, and enzymes by *Mucor pusillus* and *Endothia Parasiticia* were reported to be satisfactory. The process has been patented.[104]

The proteolytic activities in aging various cheeses are usually performed by the microbial cultures added in the beginning rather than by the addition of food enzymes. Still, a delayed action of the initial rennin takes a part in aging of cheese.

The lipolysis during the cheese aging, a flavor producing process, is usually catalyzed by the microbial enzymes produced by the specific organisms cultivated in the cheese, such as *P. roqueforti, P. camemberti*, and others. Also, unique provocative aroma and taste flavors are produced by the addition of special food enzymes, the pregastric esterases usually used in making Italian type cheeses. These lipolytic enzymes are produced from the enzyme secreting oral glands located at the base of the tongues of calves, lambs, or kids. They are called oral lipases or pregastric esterases. Examples are "Italase" and "Capalase" as listed in Table 2.

Lactase, the enzyme which hydrolyzes lactose into glucose and galactase, has more future as a food enzyme than it has had in the past. Lactose, in the form of dry milk powder, finds its way into bread and ice cream. Milk powder is used principally as a protein source. Lactose is not sweet, is not fermented by bakers' yeast (*S. cerevisiae*), and cannot substitute sugar as sweetener in foods or fermentable substrate in bread making. On the other hand, with increasing cheese consumption, lactose solution in the form of whey is produced in abundance and it has now reached the stage where it is considered a waste product in some plants, causing water pollution.

Best source for lactase is *Saccharomyces fragilis*; *Candida pseudotropicalis* also produces lactase. The yeast cells are preferably propagated in supplemented whey. Lactase, an intracellular enzyme, remains in the cells. The harvested cells may be dried (freeze-dried, spray dried, or tray dried at 45°C to 65°C) with the simultaneous destruction of zymase, the alcohol producing enzyme complex. The dried dead cells can then be used as raw lactase preparation. More purified lactase preparation can be obtained by subjecting the alive cells to autolysis and by separating the lactase containing juice from the undissolved cell residues. The cell free extract may be used as a partially purified lactase, or the enzyme may be further purified through repeated precipitation by solvents such as alcohol or acetone.

After the economic condensation of whey is accomplished, to cut transportation expenses, and a reliable and reasonably priced lactase preparation is marketed, a number of problems can be solved. Lactase, then, will be used to hydrolyze the lactose in full milk skimmed milk and whey according to the need for the diversified purposes. Far apart from food enzymes, one major problem of public interest, water pollution by dairy wastes, would be solved by an upcoming food enzyme, the lactase.

f. Enzymes in Cocoa, Chocolate, Coffee, and Candy Industries

Cocoa beans are cured to reduce the astringency of beans and to produce flavor substances. In the curing process, by the action of the native enzymes of the substrate and of the enzymes produced by the microorganisms taking part in the process, a long line of enzymes is involved. Present are amylase, protease, lipase, phosphatase, pectin esterase, polygalacturonase, catalase, peroxidase, phenol oxidase, and aminoacid decarboxylase. When the beans are sprouted the activity of all enzymes is greatly increased.

In the production of chocolate products, the added lipase plays an essential part by producing flavors from the butter and cream used in making milk chocolate and caramels. Frequently, pregastric esterases are used to modify the butter fat which contains up to 21 different fatty acids.

The complete removal of the skin and the fleshy part of ripe coffee cherries is essential in obtaining the green coffee beans. In the process the mucilaginous flesh is loosened by 24 to 48 hours of natural fermentation. Occasionally, the fermentation will produce off flavors. Experiments with commercial pectic enzymes to remove the skin and flesh were successful in one hour at 0.2% enzyme concentrations and in five to ten hours at 0.025% enzyme concentrations. The process is used in South America for making "washed

coffee." The name is given since the enzyme hydrolyzed mucilage is washed away from the beans.

Invertase, produced by *S. cerevisiae* and *S. carisbergensis*, is extensively used by the candy industry to convert sucrose into invert sugar. Invert sugar is much sweeter and more soluble than sucrose.

g. Enzymes in Extending Shelf Life

Atmospheric oxygen may adversely affect the flavor and/or color of foods and may hasten the corrosion of cans containing carbonated beverages or citrus juices. The glucose oxidase enzyme, β-D-glucopyranose acrodehydrogenase, will catalyze the oxidation (dehydrogenation) of glucose to gluconic acid with the formation of H_2O_2 in the presence of moisture and atmospheric oxygen. The enzyme is produced by *Aspergillus niger, Penicillium amazasakiensis*, and *Penicillium notatum*. The active pH range is 3-8.5 with an optimum of 5.5. At 80°C about 90% activity is destroyed within 2 minutes and at 65°C in 100 minutes. The H_2O_2 is decomposed by catalase enzyme, if its presence is objectionable. Glucose oxidase is used when the presence of ozygen or the presence of glucose is undesired.

Following are examples of practical applications:

1. The "millard darkening" of dried egg powder accompanied by some loss of flavor is caused by the small amount of glucose present, reacting with the egg protein. In the corrective treatment, the egg is first liquefied by the addition of proteolytic enzyme; then, glucose oxidase with some H_2O_2 and catalase is admixed to it. The hydrogen peroxide decomposed by the catalase supplies the oxygen needed for oxydizing the glucose.

2. Glucose oxidase added to canned beverages will prevent the corrosion of the can and the fading of the color. Glucose is supplied by the product and the oxygen by the air space. The stability of natural cloud in citrus drinks depends on the presence of cellulose. For this reason, cellulase free glucose oxidase is added to citrus drinks.

3. Mayonnaise and salad dressings become rancid in contact with air. Adding glucose and glucose oxidase to the products will prevent rancidity.

4. Dehydrated foods, such as roasted coffee, dried yeast, cake mixes, and milk powder, undergo oxidative changes in the package due to the presence of air. The oxygen is eliminated from air space by placing scavenger packets in the package. The packets contain glucose, glucose oxidase, catalase, and sufficient moisture. In the improved version the moisture is supplied in the form of agar gell. The reaction mixture is enclosed in a polyethylene film which permits the transmission of the oxygen but which will not permit the moisture to escape. Sufficient glucose is added to take up the oxygen from the air as well as that formed from the H_2O_2.

5. To prevent growth of aerobic microorganisms on the surface of cheese, the inside of the wrappers is coated with glucose and glucose oxidase.

h. Miscellaneous Food Enzymes

Anthocyanin, the pigment giving the color of red grapes, blackberries, and some other fruits, is decomposed by anthoyanase enzyme, a beta-glucosidase produced by certain fungi. Blackberries contain too much anthocyanin. To improve the color of blackberry jellies and jams, part of the color is destroyed by the addition of anthocyanase. The same enzyme may be used to make white wine from pink must or to reduce the color of dark red wines.

The bitter taste of grapefruit juice is caused by naringin, a 7-rhamnosideo α-glucoside of 4', 5,7-trihydroxyflavonone. The enzyme maringinase, obtained from microorganisms, in 0.01% quantity will debitter grapefruit pulp and juice in one to four hours by hydrolyzing naringin into prunin and naringenin.

III. ENZYME UNITS ASSAY METHOD

General Review

The "activity" of specific enzyme sources, enzyme preparations, or crystalline enzymes is measured, compared, and expressed by the amount of change it causes on a selected substrate during a given time under specified conditions. The activity is always related to the weight or volume of the enzyme. The activity of preparation "A" is twice as much as that of preparation "B" if one gram of it causes twice as much change under specified conditions as does one gram of preparation "B." The enzyme activity is synonymous with

the strength, potency, or value of a source or a product when other values such as purity, food grade, etc. are disregarded.

The enzyme "action" is the amount of change caused on a substrate. In defining the action, the amount of the enzyme and the time involved and the conditions under which the enzyme acted are disregarded. For example, in a process converting starch to glucose, the action between two points equals the amount of glucose produced. On a practical level the "action" refers to the amount of the desired product formed. The side changes are disregarded.

The "enzyme unit" is the amount of enzyme (not the preparation) which causes a definite amount of enzyme action under selected specific conditions. On a practical level the unit depends on the selected assay method; this creates the situation that several enzyme units are established for many individual enzymes.

The amount of a specific enzyme, expressed as the number of units per gram product, is determined by measuring the rate of reaction which under proper conditions is proportional to it.

Enzyme unit is defined by the I.U.B.N.C.I. as follows: "A unit is a quantity of the enzyme, related to the velocity of the enzyme reaction by an arbitrary definition, so that the velocity tells one how many units of the enzyme are present. In other words, one unit of an enzyme is usually defined as that quantity which produces a certain rate of reaction under a certain set of defined conditions."

In its effort to establish a standard unit for all enzymes, the I.U.B.N.C.I. recommended the following definition: "One unit (U) of any enzyme is that amount which will catalyze the transformation of one micromole of the substrate per minute under standard conditions."

The committee, in recognition of the difficulties confronting a uniform definition in the complexity of enzyme actions, referred to two special cases: (a) when the substrate is a large molecule (a polymer) and more than one bond is attacked, as in the case of starch or proteins, the "one molecule" in the definition should be substituted for "one micromolecule of the substrate;" and (b) in the case of bimolecular reaction (A+B=C+D) one micromole of substrate A or one micromole of substrate B can be taken as the basis; however, in the special case of a reaction between two identical molecules, when B=A, "the basis should be two micromoles of A."

At the present, we are too far from universally putting these recommendations into effect in connection with food enzymes. The recommendation still is a step forward on the way to answering the desire expressed by many enzymologists. It will pave the way for unifying values and expressions in clinical enzymology. And by a remote chance it may offer a common denominator for the future in dealing with commercial enzymes and food enzymes. Until that time, the many existing enzyme units and the even more numerous methods for their measurements will be in use for expressing and measuring the values of the various units, potencies, activities, etc.

The committee further suggested that in assays the temperature should be stated and, where practicable, it should be 30°C. The pH and substrate concentration, where practicable, should be optimal; where convenience dictates, milliunits (mu), kilo-units (ku), etc. should be used; assays should be based on initial rates of the reaction when possible; and the substrate concentration should be sufficient for saturation of the enzyme.

The committee also recommends defining "specific activity" as units of enzyme per miligram of protein in the preparation; "molecular activity," as units per micromole of enzyme at optimal substrate concentration, as the number of molecules of substrate transformed per minute per molecule of enzyme. "Concentration" of enzyme in solution should be expressed as units per milliliter.

These recommendations are more or less in conformity with the practical units, assay methods, and nomenclature. These are the main deviations:

(1) in place of micromolecules, milligrams or grams are used. The processing man got lost at the mention of molecules.

(2) In practical assay methods, the temperatures used are all over the thermometer scale. In the assays of both alpha- and beta-amylase, for instance, two temperatures are commonly used. When reporting the units the assay temperatures are also presented. It is more realistic to run the assay at the same temperature the customer is using in his process.

(3) In many instances, in defining the same assay specifications, two or more reaction times

are used by different laboratories. There are methods where the enzyme action is measured in ten minutes and the units are calculated per minute or per 60 minutes' action time. In short-cut methods using specific conditions for a specific action, in the Lasche test for example, to reach the acchromic point the time is measured and the minutes are used as units. Here, naturally, the shorter the minutes the stronger is the enzyme preparation.[29, 30]

(4) In a given assay method described at several places the pH is frequently varied, often well away from the optimum.

When the assay-time and the sample-amounts are different, the potency units still may be calculated per gram and on a per hour basis. A convenient and useful way to express the enzyme unit is by the grams of substrate hydrolyzed to a given point by one gram enzyme per minute (or 60 min) under defined conditions. This will tell the food processor how many pounds of material are acted upon by one pound of enzyme preparation within one minute. For this reason, the best way is to define the assay conditions as closely as possible to the processing conditions with the exception of the exposure time. This, in the assay method, should be short enough to fall on the linear phase of the reaction velocity.

Then the relation is expressed by the single equation:

$$\text{Units per gram} = S/Et \text{ converted} \qquad (1)$$

where

S = grams of substrate changed
E = grams of enzyme preparation used
t = minutes of exposure (predetermined or measured)

If the value of "t" is predetermined, as in many assay methods, the chemical change is measured and is expressed in grams of the substrate changed "S." For example, in the determination of the "Saccharifying Power (Lintner value)" of an amylase preparation, the amount of reducing sugar produced from starch in 60 minutes is measured.

If the amount of change is predetermined, for example, in measuring the Lasche time, the alpha-amylase content of an amylase preparation in measuring the milk clotting power of rennin or of another proteolytic enzyme, the time is measured.

In both type assays the U/g=S/Et may be used as a basis for calculating the units.

In order to show high unit values, the units are defined per hour: the values obtained per minute are multiplied by 60.

Measurements of enzyme activity, shortly enzyme assays, to determine the enzyme units contained in a gram preparation are described in various publications:

Methods of Enzymatic Analysis (1965)[29] is the most comprehensive recent book on the subject. The book edited by H. W. Bergemeyer, 2nd ed., (Verlag Chemie GMBH Weinhein, Germany) Academic Press, New York, on 1064 pages in four sections and 15 chapters written by 117 authors presents the most comprehensive information on analytical methods connected with enzymology. In the first 654 pages, methods are given with respect to enzymatic reactions organized according to the substrates and their metabolism, such as (1) carbohydrates, (2) substances of the citric acid cycle, (3) proteins, (4) fatty acids, lipids, and steroids, (5) nucleosides, purines and pyrimidines, coenzymes and related substances, and (6) miscellaneous substances.

On the last 423 pages the measurement of the enzyme activity and the use of enzymes as reagents are discussed as organized according to the group of enzymes. In this part the enzymes are grouped as 1. Aldolases such as fructose -1, 6-diphosphate aldolase and 1-phosphofructoaldolase; 2. Dehydrogenases, such as lactic dehydrogenase, glucose-6-phosphate dehydrogenase, malic dehydrogenase, etc.; 3. Esterases such as cholinesterases, lipase, phosphatases, glucose-6-phosphatase, ribonuclease, etc.; 4. Proteases; like trypsin and chymotrypsin, pepsin, peptidases; 5. transaminases; 6. Other enzymes such as amylase β-glucosidase, glutathion reductase (TPNH and DPN specific glutathion reductases), catalase peroxidase, reductases, invertase, urease, xanthine oxidase, etc.

Toward the end, the chapters on hystochemical detection of enzymes and on biochemical reagents are presented with great competency.

The bibliography is given on the individual pages in footnotes where the text contains references.

This very thorough and remarkable book is a must to be read by any student of enzymatic analysis and assay method. It presents all basic concepts of enzyme assays and detailed descriptions of hundreds of assay methods.

For example, trypsin is discussed on 12 pages,

including detailed descriptions of three assay methods. For each method the following are discussed: (1) principle, (2) optimum conditions for measurement, (3) reagents, (4) preparation of solutions, (5) procedure, (6) calculations, and (7) examples.

Two of the described methods contain the very thorough description of the "hemoglobin" and "casein" methods; the third assay uses benzoylarginin ethyl ester as substrate.

In the "biochemical reagents" section, 40 purified enzymes are characterized, giving their molecular weight, crystal form, the reaction they catalyze, the activators, inhibitors, chemical properties, activity measurements, stability, purity, and the producers.

This book is nearly the enzymologist's dream. Still, a process control man of a food processing or food enzyme manufacturing plant, who will study it for hours or days to pick up an assay method he needs badly, will be disappointed more frequently than satisfied. No guidance or critical evaluation of the methods is given. He will feel as a child left alone in a forest. There are too many trees; he will not see the forest. He will go back to the AOAC book, or the NF (National Formulary) book or the USP (U.S. Pharmacopaeia) and The Cereal Laboratory methods, and in final desperation he will try to design a new method of his own having roots in all the books he consulted.

This situation is not the fault of the Bergmeyer book nor of the process control chemist. It is the gap between them and their purposes and needs. The brilliantly executed book is aimed to serve all kinds of enzymologists, principally the scientific type, where factuality and precision overrule everything else, including time or expense. The man with process control problems in food enzyme assays, too, is brilliant, intelligent, and well educated. But he is confronted with production managers with strict time schedules on one hand and with cost-conscious accountants on the other. He is looking for assays that may be executed quickly and at a minimum labor cost. He needs short-cut methods, simple enough to be carried out by laboratory assistants, which never fail and still give the information the production manager needs.

He will find no assay methods, for example, for invertase, maltase, or latase in the Bergmeyer book, although five pages are devoted to the assay of raffinose using invertase as reagent and four pages to the assay of lactose using lactase as reagent.

Practical assay methods and enzyme units concerning pharmaceutical enzymes (digest aid) are published in the U.S. Pharmacopaia,[5] the National Formulary,[5a] and the United States Dispensatory.[132]

Assay methods and enzyme units for food enzymes are presented in the A.O.A.C. [65] The Cereal Laboratory Methods[7] and the Methods of Analysis of American Society of Brewing Chemists.[4]

These methods are the products of devoted individual scientists and of committees composed of experts. They all have their merits and shortcomings. Merits are indicated by their acceptance and applications and their shortcomings by the many modifications and recommendations for new assays published constantly. It is customary to improvise a new assay method for any major research work where enzymes are involved to satisfy the specific need or to emphasize the main objective of the research.

For their own use and for their customers the major manufacturers of food enzymes adapted the most dependable published methods. In a few instances they are identical with those given in official publications. They are, however, often simplified or revised. The manufacturers generously supply the assay methods to their customers and to interested enzyme scientists.

To illustrate the characteristics of the various methods and enzyme units the following examples are listed:

2. Carbohydrases

α-Amylase

The unit is the amount of enzyme which will dextrinize one gram soluble starch in the presence of excess β-amylase at 20°C in 60 minutes.

The assay method, standardized by the American Society of Brewing Chemists, 1958,[4] is adapted by the Association of Official Analytical Chemists (AOAC) and Cereal Chemists. It is also reviewed in refs. 32 and 33.

The number of α-amylase units in one g enzyme preparation. =

$$\frac{\text{g starch hydrolyzed} \times 60}{\text{g of malt} \times t}$$

t = reaction time determined in the assay at 20°C.

α-Amylase, the starch liquefying enzyme of

malt, is essential in processes utilizing malt enzymes to liquefy gelatinased starchy paste, such as in corn syrup and glucose production. Good distillers' malt contains up to 50 α-amylase units per gram and Gibberellin treated malt up to 100 units. Some laboratories digest the starch at 30° C, yielding much higher units per gram.

Both the unit and the assay method are typical, or almost classical examples. The calculation satisfies Equation 1. Still there are two factors which are far from the conditions of practical applications; the nature of the substrate and the temperature used.

The most desired action of alpha-amylase is the liquefication of gelatinized starch. The assay method, as do most amylase assays, uses soluble starch (Merck according Lintner). This is for convenience and for having a uniform standardized substrate. Viscosimetric methods developed early this century, with untreated starch for substrate, are used only in special cases, often to measure the liquefying activities of bacterial enzymes.

In practical applications alpha-amylase is permitted to act at 55 to 60° C (optimum 60 to 62° C). There is a chance that some of the trace amylases, always present in an unpurified enzyme preparation, may act in the assay at 20° C but become inactivated at 60° C.

Nevertheless, the α-amylase assay is popular, and the figures obtained are dependable to compare alpha-amylases of different sources and to use as a tool for process control in the manufacturing of alpha-amylase preparations as well as in their utilizations. The method is used extensively to evaluate malts and malt preparations.

"Lasche Test"[29, 30]—In a quick and simple operation this test measures the minutes necessary for 0.55 g enzyme preparation (malt) to convert 4.4 g soluble starch to the acchromic point at 62° C under specified conditions. The time in minutes is used as an index for the evaluation of malt amylases (principally α-amylase) and may be substituted into Equation 1. The calculated units obtained can be used for linear comparison of the α-amylase content.

Example: Malt Sample A = 4 min Lasche time
Malt Sample B = 8 min Lasche time

$$\text{A.}\quad C = \frac{4.4 \times 60}{60 \quad 0.55 \times 4} = 120$$

$$\text{B.}\quad C = \frac{4.4 \times 60}{60 \quad 0.55 \times 8} = 60$$

C = "Converting capacity" proposed by de Becze et al.;[30] "60" indicates 60 minutes' action time (digestion time).

Starch Dextrimization "SKB" Unit

The SKB unit defined by Sanstedt, Kneen, and Blish[102] equals the amount of enzyme that dextrinizes (converts to acchromic point) 1 g β-amylase treated starch in one hour at 30° C and pH 4.85. The method is designed to measure the SKB units contained in one g malt.

All three methods above are the modifications of the old Wohlgenuth method developed toward the end of the last century. The first method is based on the SKB method.

Starch-Liquefying Unit[32]

A 1000 liquefying unit will reduce by 90% the viscosity of 300g potato starch or 560g tapioca starch in ten minutes at 70° C and pH 6.7. The method, as a practical assay, is used by manufacturers of starch syrups. Both the temperature and the pH are well above the optimums for malt enzymes.

Diastatic Power or Saccharifying Power ("Lintner Units" or "Degrees Lintner.")

The assay measures the β-amylase content of an enzyme preparation, mostly of malt.

A preparation has 100 units "Saccharifying (diastatic) Power" if 0.1 ml of a clear 5% infusion of the preparation acting on 100 ml of a 2% starch solution (Merck) at 20° C for one hour produces sufficient reducing sugar to reduce completely 5 ml of Fehling solution. Details of the method, used in breweries and bakeries, is given in detail.[4, 65]

Limit Dextrinase

One unit equals the amount of enzyme which produces one milligram fermentable sugar from standard limit dextrin solution in one hour at 30° C under specified conditions.[32] The method is used in studying and comparing enzyme qualities of preparations or natural substances of different origin.

Diastatic Activity of Flour

The unit equals the amount of enzyme producing 1 mg maltose in 1 hr at 30° C under specific

conditions. The starch content of the flour is the substrate.

In the assay, 5 g flour is digested at 30°C for 1 hr after being mixed with a teaspoon of ignated quartz sand and 46 ml acetate buffer solution. At the end of 1 hr of digestion the enzyme is inactivated by 2 ml 0.1*N* H_2SO_4. After clarification with 12% $Na_2WO_4 \cdot 2H_2O$ solution the digest is filtered. The maltose content is calculated from the reducing power determined by the ferricyanide method.[65]

The method is used in the milling and baking industry and in cereal research.

Invertase[32, 34, 135]

One invertase or saccharase unit is equal to the amount of enzyme which converts 20 ml of 20% sucrose solution to 0° rotation at 20°C in 1 hr. At 0° rotation, 75.93% of the sucrose is hydrolyzed. This assay is tailored to take advantage of the easily adaptable polarimetric method.

Methods based on reducing sugar determination are also in use.

Maltase, Lactase, Inulinase[96, 135, 152]

Reference books of the last two decades ignored presenting units or assay methods for these very common enzymes. Probably, they assumed every enzymologist had devised one, based on measuring the change in optical rotation or in the reducing capacity of the substrate digested with the enzyme under specified conditions for a specified time period. Such omission of practical methods for common food enzymes is only possible because "food enzymes" as such had not yet reached the status of recognition as a "group."

3. Proteinases

Proteinase Unit for Papain[65]

One unit equals the amount of enzyme that produces a titration difference of 1 ml 0.1N KOH under conditions specified in the casein test.

In the test, 10 ml of 6% soluble casein is digested for 20 minutes with the activated extract of 10 mg enzyme preparation at 40°C and pH 5. The enzyme is activated by a half saturated H_2S-H_2O solution for 1 hr at 40°C.

Pepsin

A proteolytic enzyme prepared from the glandular layer of hog stomach, it should digest not less than 3000 times its weight of coagulated egg albumin.

In the assay, 10 g of freshly boiled and minced egg white (albumin) are digested with 5 ml of a solution containing 0.1 g pepsin in 150 ml (diluted HCl solution) at 52°C for 2.5 hr under frequent agitation. In parallel sets, egg whites are digested with NF standard pepsin solution.

The corresponding values of the undissolved egg albumin residues are compared in NF-specified volumetric flasks. Details of the test are given in ref. 132. Results of the two tests should not be less than in the test of NF standard.

Pepsin and Rennin Elixir

The elixirs of pepsin or rennin in 100 ml should possess a proteolytic activity not less than 2.25 g reference NF pepsin.[5a] The elixirs are assayed by a test similar to the one used for pepsin.

Rennin Standard

The milk-curdling enzyme obtained from the glandular layer of the fourth stomach of calves should contain not less than 90% and not more than 110% of the NF reference rennin.[5a]

100 mg samples of the standard and of the unknown are each extracted separately with 50 ml water for 15 minutes. In two flasks, 50 ml cows' milk brought to 43° C is kept at that temperature in a water bath. One ml of the standard extract is added to one flask and one ml of the unknown to the other. The time is measured when the thickening in each flask is noted. A convex surface is formed by the milk if the flask is tipped to a 45° angle when the milk is curdled.

Hemoglobin Units–HU

A preparation has 1000 HU per g if 11.18 mg of it produces a 500 mg increase in soluble nitrogen from 0.417 g of hemoglobin, in five hours at 40°C and pH 4.7.[65]

Proteolytic activity of flours and malted wheat flours is expressed in HU per g sample.[65] In the assay, 2.5 g bacto hemoglobin is digested in 50 ml acetate-buffer (pH 4.7) by the sample at 40°C for 5¼ hours. From flours with low activity a 10 g sample is used per test; from active samples, an extract is first prepared and an exact portion is used. The difference in the solubilized nitrogen content of the digested sample and of the control is the result of the enzyme action. From this value the HU is calculated.

Casein Solubilization Unit

According to a commercial definition,[32] a preparation containing 1000 casein solubilization

units will solubilize nine times its weight of casein in one hour at 40° C and pH 8.

Northrop Unit NU

The unit equals the amount of enzyme which hydrolyzes 40% of 1 mg casein in 60 minutes at 40° C and pH 8.

Several variations of the assay measure the amount of tyrosin produced.

The spectrophotometric method measures the OD at 275 mμ. The OD is standarized with pure tyrosine at this wavelength.

In the photometric method, a blue color is formed in the digest by the addition of Folin Cicocalteu (phenol) reagent. The color intensity is measured at 660 mμ wavelength. The light transmission or the OD is standardized with pure tyrosine and phenol reagent.

The Marschal Division of Miles Laboratories, Inc.,* using the spectrophotometric method, defined its own unit, MDU (Miles Detergent Unit), as the amount of enzyme required to liberate 10 nano moles of tyrosine/min under the conditions of the assay. One nano mole is equivalent to 1×10^{-3} micromoles.

In recent years the Northorp unit, originally used for papain and bacterial proteases, has been adapted for assaying detergent proteases following the European and Japanese examples.

4. Lipolitic enzymes (Lipases)

Triacetin Unit (TAU)

One unit is equal to the amount of enzyme which produces 1 ml of 0.1N acetic acid in 1 hr at 50° C and pH 5.5 from 1 ml of glycerol triacetate (triacetin) dissolved in 25 ml of water. The method is described in ref. 32.

Lipase Commercial Unit[32]

The unit is defined as the quantity of enzyme which will liberate one milliequivalent of fatty acid from olive oil emulsion in 2 hr at 37° C at 7.3 pH.

5. Pectic Enzymes

Pectase (Pectin Methyleasterase) Units

Following are the definitions of three different units:

1. One PMU equals the amount of enzyme that splits off 1 mg methoxyl in 30 min at 30° C and pH 6.0.

2. One PEU equals the amount of enzyme which at 30° C and optimum pH will catalyze the hydrolysis of one milliequivalent ester bond per minute in the presence of 0.15 N sodium chloride. One PMU equals 930 PEU.

3. The third unit equals the amount of enzyme that will liberate 32 mg of methanol from pectic acid dissolved in 0.1 M phosphate buffer solution in 1 min at 20° C and pH 8.0.

Pectin Galacturonase Unit

The unit equals the amount of enzyme that will hydrolyze 1 mg of polygalacturonide at 40° C in 1 hr. In the assay method, pectic acid is used for substrate, and the increase of the reducing power is measured.

LITERATURE

Many aspects of food enzymes are frequently discussed in some detail in every study on enzymes. The rapid development of food technology and the prominent position of food enzymes within the last few decades inspired two outstanding recent reviews.

Reed's book,[96] Enzymes in Food Processing, a monograph of Food Science and Technology, was published by Academic Press in 1966. On 483 pages in 20 chapters and 3 appendices, the author discusses in detail all essentials of food enzymes. The book covers the sources, nature, characteristics, kinetics optimum conditions for activity, and inhibition and activation of enzymes. It presents the individual enzymes in four groups: carbohydrases, proteolytic enzymes, lipases, and oxidoreductases.

Separate chapters deal with the food enzymes applied by individual industries as follows: milling and baking, starch syrups and dextrose, dairy industry, fruit products and wines, distilled alcoholic beverages and beer, meat and other proteinaceous foods, candy, cocoa, chocolate and coffee flavors, and miscellaneous uses. The chapter on the production of commercial enzymes by Underkofler (pages 197–220) is a worthy contribution to the book. Appendix A gives an insight into the cost of food enzymes. More than 1,000 references are given.

The other excellent review on food enzymes by Underkofler was published in the *CRC Handbook*

* Miles Laboratories, commercial pamphlet

of Food Additives[57] in 1968, edited by Thomas A. Furia. The 86-page review (chapter 2) in a clear, concise style briefly discusses the nature and theory of enzymes, their names and classifications, modes of action, methods of assay, and the management of enzymatic action. It covers the utilization of enzymes in the manufacture or treatment of dairy products, meat, cereals, vegetables, and fruits. It further discusses the industrial enzymes and their applications according to various classes, such as carbohydrases, pectic enzymes, proteases, lipases, glucose oxidase, and catalase. The 201 references add to the value of this highly informative chapter.

The longtime experience in the field of industrial enzymes, including food enzymes, of these two authors and their experience and skill in technical writing make their contributions invaluable for the newcomers to the field and to the young students who wish to make a lifetime career dealing with food enzymes.

The large number of publications of food enzymes according to their types may be grouped as follows:

(1) Advanced news items on a new enzyme or on a new application of an old enzyme usually published in the news edition of scientific journals, trade magazines, or daily papers. They bring news about the potential replacement of an old and established reliable product by a new enzyme obtained from a different (usually microbial) source, with potentially added effects and for lower cost. These news releases, usually appearing as editorials, although in substance they are probably paid advertisements, are strongly suggestive and commercially oriented with frequent exaggerations by the writers and the editors. Still, they often contain accurate and pertinent information the literature researcher can hardly find in other places.

(2) Joint publications by leading personalities of various manufacturing companies satisfy the need for information and clarify some gray areas for the benefit of their customers. Such jointly authored publications are excellent sources of technical information. Their contents usually constitute infused general knowledge. But the articles signed by established names add emphasis and credibility to their content and establish the position of the industry in disputed points. They are milestones in the train of technical developments. The authors, having scientific minds and training, cannot help but present the information with scientific integrity and to add a little more than the reader could learn from other sources or could suspect by educated guessing. Such joint articles, aimed to promote both customers and academic education, are censored and approved by the managements of the companies involved.

(3) Similar in quality and purpose to group 2 are publications written by research executives of individual companies. They are market oriented and frequently aim to establish priority in some innovation: new product, new field, or new technique in application. Frequently, they are related to a pending or granted patent. Both types 2 and 3 usually appear in scientific journals, occasionally in trade journals.

(4) Patents are usually excellent sources of information on processes producing and utilizing food enzymes. Patents may be granted to industrial researchers as assignors of their employers. Many valuable patents are obtained by the U.S. Government on processes developed in the regional laboratories of the Dept. of Agriculture. Industrial patents aim to prevent others from using the method; government patents aim to prevent exclusive rights for a single individual or corporation to monopolize a useful process. Government owned patents are public properties available for everyone in the U.S. free of cost. Only a formal license is needed.

(5) Special research reports, usually presented with high competence and at a high scientific level, written by scientists of the U.S. Government (usually in the Dept. of Agriculture) and by faculty members of universities, give excellent details and scientific explanations on the development of food enzymes, their evaluations, and applications. The references they list are frequently helpful for other researches.

Research at universities is often sponsored by industrial concerns. It has been remarked that a successful research ends up in a patent application; the luck of practical value may increase the number of scientific publications. Nevertheless, the descriptions of experimental techniques, equipment, and assay methods used, usually well illustrated with mathematical treatments, given in research reports coming from universities are the best in the field. They keep the knowledge on food enzymes at a scientific level, and sometimes they exercise a quick check on the factuality of the commercially oriented literature.

True, quite often such reports expect too much preliminary knowledge and overemphasize side issues, such as mathematical or statistical treatments of their otherwise mediocre or insignificant findings.

(6) Rulings and regulations by the FDA of the Dept. of Health, Education, and Welfare are valuable in establishing facts, and they do outstanding services to the public interest. Since they are born through strong debates offered by both the food and enzyme industry and are formulated by experts, such regulations and rulings are sources of fundamental facts.

(7) Manufacturers pamphlets, frequently luxuriously, executed, aimed for customers' education and acceptance, are usually factual and highly informative. Written by scientists for processing engineers, they use simple, direct language in a clear, concise manner. They are reliable sources on pH and temperature optimums and ranges, thermal inactivation points, and on activators and inhibitors. Frequently, they disclose the source of the food enzyme, even giving the name of the organism used. They specify enzyme-substrate ratios, substrate concentrations, action times, substrate-product equilibriums, along with fairly good scientific background. They are factual as well as businesslike.

The sales pitch in such writings may exaggerate the inimitable achievements and the full satisfaction obtained by using the product. As a natural and understandable part of such pamphlets, it is disregarded by the students of food enzymes and accepted by the customers only after several successful applications of the recommended product.

In some instances, the "food product" produced by enzymatic actions is advertised; for example, glucose syrups or fructose made from glucose by enzymatic action, etc. From the composition of such products, made by the action of commercial enzymes in highly secretive processes, certain conclusions can be drawn on the nature of the enzyme and its action.

(8) Manufacturers' instructions for assaying food enzymes, usually selected from top scientific methods and streamlined to down-to-earth practicability, are, in general, the most realistic and reproductable methods, satisfying practical aspects. High priced products are sold and purchased based on those methods, and sensitive processing methods are controlled by the results following those assay instructions. Many of them are uniformly accepted by both the enzyme and food industries and are frequently standardized by joint committees composed of expert scientists.

(9) Assay methods are standardized and published by scientific, official or semi-official, and trade institutions and societies, such as the U.S. Pharmacopeia, National Formulary, AOAC, Association of Cereal Chemists, Association of American Brewing Chemists, etc.

These assay methods, published in manuals of the various societies, vary from highly sophisticated, often outmoded, methods to simple short-cut tests to satisfy the customer's process control or, occasionally, the specific trade interests of the manufacturer of the food enzyme. One of the fields in food enzymes that needs a thorough revision is the definition of units and restandardization of the assay methods.

In the AOAC book detailed descriptions are given of assay methods for the following food enzymes: diastatic power (β-amylase), alpha-amylase, diastatic activity of flour, proteolytic activity of papain, phosphatase, and residual phosphatase. Since the AOAC methods are used by all official analytic chemists and their findings stand up in courts, these methods deserve special attention. They are principally designed to assay enzyme activities in specific foods or food substances, such as milk, milk products, flour, malt, and malt products. These methods have been standardized by the AOAC using assays based on old U.S.P.,[5] National Formulary,[5a] U.S. Dispensatory,[132] and Cereal Laboratory methods.[7] They served the needs of the past, but they can hardly satisfy the present requirements; and, for sure, they have not much future.

These methods are over 30 years old and in many respects are below the level of the needs of the present time. The definitions of the units, wherever given, indicate the time period when the methods were designed and/or adapted. They lack uniform treatment. The reevaluation, restandardization, simplification and, where possible, automation of the assay methods is becoming more imperative every year. The thorough revision of the assay methods of food enzymes and enzyme units should be the first project of the evolving new branch, food enzymology.

Also, food enzymes should not be listed, considered, or discussed with enzymes used for designing textiles, curing leather, or cleaning

laundry even if the active substance and the mode of chemical actions are identical. The difference in the purpose and importance between food enzymes and other industrial enzymes is great. All dealings and considerations regarding enzymes are organized by and centered around the purpose they serve rather than the chemical reactions they accelerate.

Pertinent scientific and technical articles, news items, editorials, paid advertisements, and trade promotional articles dealing with food enzymes are published (not exclusively) in the following journals: *Chemical Engineering, Chemical and Engineering News, Journal of Agricultural and Food Chemistry, Cereal Chemistry, Cereal Science Today, Journal of the Association of Official Analytical Chemists, Journal of Society of American Microbiologists, Food Engineering, Wallerstein Laboratories Communications, Food Processing, Chemical Week,* and *Technology Review.*

Titles and Dates, Examples

The scope of this article and the time the reader can afford to read it will not permit reviewing even a small fraction of the recent publications dealing with food enzymes. In place of a systematic review, a few publications picked up at random will be referred to as illustrating examples on what is being published. Short comments will be given as examples on the reaction of the average reader with general interest on food enzymes. These illustrations aim to alert both the writers and readers to what kind of reactions their publication will face and what kind of publications are offered to read. Titles and dates of publications carry much weight.

1. Frenzy Over Enzymes, an editorial on March 15, 1969, *Chemical Week*. Both the title and the eye-catching colored illustration of the market estimation for 1968 vs 1972 on the enzyme market for food, beverage, industrial, drugs-miscellaneous, and home laundry products capture the interest of both the market and the enzyme oriented reader. He is not disappointed reading the artfully condensed professional editorial. He learns that one half of the $40 million per year enzyme market in the U.S. goes to food enzymes; soon the laundry enzyme consumption will amount to 15 million pounds per year (about $60 million in addition to the $40 million); there are 15 to 20 interested companies entering into the race of producing laundry enzymes; two big names, Pfizer and Monsanto, are out in the lead.

The leading products (bacterial proteases) are "Alcalase," produced in Denmark by Nove Industries and marketed in the U.S. by Enzyme Development Corp. (New York) and "Maxatase" produced by the Holland Royal Netherlands Fermentation Industries, who licensed the manufacturing process to Pfizer in the U.S. The race for the laundry enzyme producers became too crowded, and only a handful has a chance to stay. The traditional big three are Miles Laboratories, Rohm and Haas, and Wallerstein Laboratories; and there are a half dozen who are equipped to make laundry protease. In addition to these, a number of other interesting facts are jammed into the artfully loaded news item. The boom in laundry protease will stimulate progress in food enzymes.

The reader, satisfied with the two minutes' time he spent, becomes filled with interest and many questions, just as the writer expected. A year later, today, he knows much about what happened, but the list of questions in his mind concerning "frenzy over enzymes" is still growing. One of his questions could be, how much time did the staff writer spend researching his material and then jam it into one page? It could be a week or two or three. . .its biggest hit was the timing: it was printed at a time when all enzyme technologists watched the auctioning of the big show.

2. "Pure" and "Dirty" Research,[93] in the Call for Pepper and Salt section of August 1968, *Food Technology* is the shocking title of an excellent "short short," comparing "theoretical" and "applied research" with the following conclusions: "The aim of our professional organizations, educational institutions, and research centers should be to make their greatest possible contribution to excellence, and to the human race." This beautifully exalted conclusion rewards the reader for his effort in reading the one-page article written by an outstanding food enzymologist. The reader, shameful for himself, is somewhat disappointed by the noble level of the text. He expected more laundry cleaning and discord in the text under the extremely provocative title. And for his conclusion, he would say, "I wonder if the author did put all of what was in his mind when selecting his title." Here, both the title and the conclusion were the hits.

3. The Story of Rennin Substitute Informative and effective titles of news items, articles,

patents, and other publications and their date and place of publication often tell interesting stories of new developments. Following are examples:

Beridge, N. J., Renin and the clotting of milk, *Adv. Enzymol.*, 15, 423, 1954.[15]

Milk clotting activity of pepsin and rennin, *Milk Prod. J.*, 52 (5) 8, 1961.[54]

Fungal milk clotting enzyme for cheese manufacture, Japanese Pat. 15,268, 1962.[79]

Milk coagulation enzyme "microbial rennet" and methods of preparation thereof, U.S. Patent 3,151,039, 1964.[10]

Proteolytic activity of crystalline rennin and casein associations, *J. Agr. Food Chem.* 13, No. 5, 414, 1965.

The clotting of milk by proteolytic enzymes, *Biochim. Biophys. Acta*, 97, 159, 1965.[37]

Milk curdling enzyme elaborated by endothya parasitica, U.S. Patent 3,275,453, 1966.[104]

Cheese Making Enzyme Replaces Rennet II., *Food Eng.*, 39, 88, May 1967.[44]

Pfizer markets enzyme to replace rennet, *Chem. Eng. News 45*, 54, March 27, 1967.[51]

First practical substitute for animal rennet in cheese making, *Food Process. Marketing*, May 1967.

Produce rennet-like enzyme from vegetables, *Food Eng.*, 41, 119, Aug. 1969.[52]

The titles and dates suggest the outlines of the emerging of a new food enzyme. But if the reader would as much as skim the publications to register a few key words or to read the summaries, wherever presented, the outlines will turn into an alive drama of concentrated research and development work to satisfy the need for replacing rennet, and of a race against time to take advantage of a market opportunity. He will see how public interest was kept alive by dishing out information piece by piece until the final accomplishment is evidenced, accepted, and approved.

By 1955 the mode of action in milk curdling by rennin was well explored. In 1965 the two French researchers in a classical paper gave a detailed account of the happenings when rennin works on κ-casein. The reader, however, will record the little remark that by that time there was no satisfactory substitute for rennin. In the same year Dennis and Wake explored milk clotting by proteolytic enzymes other than rennin, following the footsteps of Ernstrom in 1961.

Already in 1962, however, a Japanese patent was obtained for milk clotting by fungal enzyme, and in 1964 a U.S. patent was granted to two Japanese researchers for the preparation and use of "microbial rennet." In 1966, Sardinas obtained a U.S. patent for an enzyme produced by *Endothia parasitica* for curdling milk.

The one-page commercial editorial in the May 1967 issue of *Food Processing and Marketing* summarizes the essentials. Source of rennet extract is decreasing since fewer calves are slaughtered and calves are being fed at an early age with a diet containing little or no milk. An enzyme preparation produced by the use of a strain of *Endothia parasitica*, manufactured by Chas. Pfizer & Co. and marketed under the name of "Sure-Curd" has proven satisfactory to replace rennet. The Federal Standards of Identity, effective March 15, 1967, permitted the use of the new enzyme in the production of washed curd, cheddar, colly, granular, and Swiss cheeses.

The news item is illustrated by three pictures: one shows that five relatively small jars of the powdered new enzyme equal a five-gallon single strength animal rennet extract; the other picture shows pouring of the liquefied "Sure-Curd" (about 10 gallons) into a large curdling tank filled with milk; the third shows a culture collection with the caption that the hundreds of microorganisms leading to the new enzyme were selected from 19,000 cultures. The pictures and their captions do the exaggerations. But if "Sure-Curd" will work to full satisfaction, the reader learned a lot in a minute and half. He also realizes that the organism in Sardinas' patent and the one making "Sure-Curd" are the same and that the new enzyme is not called rennet.

The two-page (semi-commerical) editorial, "Cheese Making Enzymes Replace Rennet," illustrated with two pictures and a graph, published in the May 1967 issue of *Food Engineering*, confirms

and complements the information given in the former news item. It reports that the price of rennet in recent years ranged from $7.00 to its current $20.00 per gallon; the many vegetable and microbial enzymes tested to substitute for rennet failed and only pepsin is suitable for partial replacement. The enzyme "Sure-Curd" produced by *Endothia parasitica* in performance is identical or very close to rennet; the aging of cheddar is faster when made with "Sure-Curd" than when made with animal rennet; and experiments to replace rennet in Monterey, Munster, Linburger and various Italian cheeses are successful.

The reader concludes that developments in the feed industry—taking the milk away from the calves and feeding them with something else—reduced the natural source of rennet; that, again, microorganisms come to the rescue of the situation; and that there is a new enzyme, not quite identical with rennet, waiting for a short, popular name. He cannot fail to observe the speed with which Pfizer is moving into the enzyme field. He will conclude also that the classical story of rennet and its replacement has not yet come to an end; identical or nearly identical enzymes usually are produced by more than two specific types of cells.

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I wish to express my appreciation to the following individuals for providing useful information and literature on industrial food enzymes and their assay methods: Dr. L. A. Underkofler and Mr. W. G. Allen of Miles Laboratories, Mr. R. S. Lesniewski of Chas. Pfizer Co. Inc., Mr. George Motoasca of S. B. Penic Co., Mr. G. E. Clark of Rohm and Haas Co., and Dr. L. M. Waldt of Wallerstein Laboratory. Their contributions provided an insight into food enzymes and their evaluation as the manufacturers see it.

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