Biotechnology

A Comprehensive Treatise in 8 Volumes
edited by H.-J. Rehm and G. Reed

Volume 5
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Food and Feed Production with Microorganisms

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Preface

This volume combines a description of all food fermentations. The purpose of these fermentations is to produce or preserve foods or to make them more palatable, more nutritious, more digestible, or a combination of these properties. In addition food fermentations can be distinguished from all other fermentations by the following, important characteristics.

The origin of these fermentations can usually be traced to antiquity but no specific time period or place of origin can be assigned. All food fermentations are spontaneous or "natural" fermentations, or they began as "natural" fermentations; and all of them show a development from home or communal production to industrial production, at least in the developed countries.

Food fermentations are not pure culture fermentations. The substrates are sometimes pasteurized but never sterilized. The cleanest substrates are probably ethanol for vinegar production and boiled brewer's worts. All food fermentations are mixed culture fermentations comprising not only different strains of microorganisms but different species and genera of yeasts, fungi, and bacteria. Spontaneous fermentations usually show a succession of organisms. For instance, the fermentation of soda cracker sponges begins as a yeast fermentation and ends as a lactic acid fermentation, or the wine fermentation which starts with a succession of yeasts (from less ethanol tolerant to more ethanol tolerant yeasts) and ends often with a malo-lactic fermentation. In many instances the fermentation is carried out entirely by the natural microflora of the substrates or by inoculation of subsequent batches of a fermented food with a retained portion of the preceding batch. This is particularly true of the indigenous fermented foods treated in Chapter 11. On the other hand, several foods are fermented by massive inoculation with a specific organism that has been produced separately for this purpose. Examples are the production of white bread with baker's yeast or the production of wine in which the native microbial population has been suppressed by the addition of sulfur dioxide followed by inoculation with a desirable wine yeast.

In the brewing industry it is possible to prepare a brewer's wort that is almost sterile and to inoculate it with pure culture yeasts. However, the fermentation cannot be carried out under the stringent pure culture conditions that prevail, for instance, in the production of antibiotics. In industrialized countries the trend is towards massive inoculation with desirable strains of microorganisms, so-called starter cultures, produced in separate facilities and often by separate companies.

There are several food related fermentations which have been included in Volume 3 of "Biotechnology", "Biomass, Microorganisms for Special Applications, Microbial Products". These are the production of edible microbial biomass, the production of baker's yeast, of edible mushrooms, of starter cultures for milk and meat processing, of starter cultures for other purposes, and the production of ethanol, acetic acid, lactic acid, citric acid, amino acids, and of extracellular polysaccharides. The production of baker's yeast and of lactic acid starter cultures has the most direct impact on the production of fermented foods and the reader should consult the appropriate chapters.

It is difficult to understand the role of microorganisms without some knowledge of the technology of fermented foods. Therefore, the chapters in this volume treat both the technological and the microbiological aspects of fermented foods. It is hoped that a proper balance between processing information and the microbiological aspects of food fermentations has been achieved.

It is foreseeable that in the near future the novel techniques of genetic engineering will permit the creation of microorganisms with properties especially desirable for food and feed production. Some important results through genetic manipulation have been achieved recently in the production of certain pharmaceuticals and selected pure compounds as referred to in Volume 6a of "Biotechnology", but little progress has been reported so far from the food and feed industries. Hence, the present volume is rather "classical" in this respect. Some novel achievements such as the application, in the sugar industry, of "synthetic" varieties of *E.coli* or of α-galactosidase isolated therefrom will be reported later (Volumes 6b and 7).

The authors of this volume have worked diligently to make their wide knowledge and expertise available to the reader. The value of this comprehensive volume rests basically on their contributions. The volume editor also wishes to thank Prof. H.-J. Rehm for his invaluable editorship and Dr. H. F. Ebel and Christa Schultz of Verlag Chemie for their skill and dedication in getting this volume into print. The members of the Editorial Advisory Board of "Biotechnology" contributed much useful advice.

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Gerald Reed

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Baked Goods

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I. Introduction

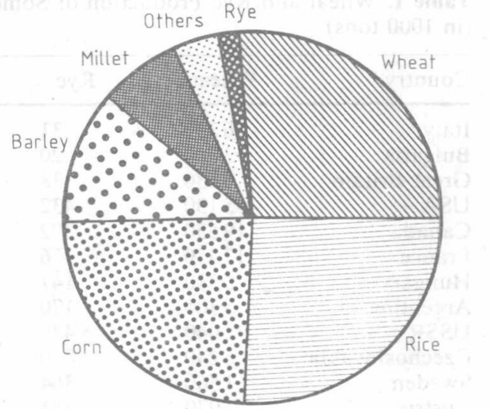

Figure 1. Production of various cereals worldwide (calculated from data of the FAO Production Year Book by ROTHE, 1980).

Cereals and bread have been the basic food for individuals and population groups for many centuries. In various countries bread accounts for 18–80% of all nutrients.

The history of bread can be traced back about 6 millenia. It is probable that it developed from a gruel. During the earlier stone age the preparation of a gruel made from rubbed or ground grain and water or milk was known by all civilizations. The flat bread developed from this gruel which was either air-dried or baked on hot stones. The first primitive baking was done by placing formed doughs into hot ashes.

Towards the end of the stone age the flat bread had assumed the shape of a disc. These breads were generally eaten warm, or they could be dried and stored. All types of grain could be used for the preparation of the gruel, but the choice of grains for the preparation of baked flat breads is narrower. Therefore, flat breads were introduced only slowly and only in some localities. Barley and other grains (hardly known today) were used. With the change to the loaf bread the choice of suitable grains is still narrower. Today wheat is grown worldwide for this purpose (Fig. 1, Table 1). Beyond this rye is grown in Central and Northern Europe. Wheat and rye are also called "bread" grains because of their suitability for the production of baked goods.

The production of a loaf bread presupposes the development of the baking oven and the discovery of the dough fermentation. The earliest baking oven has been found in the area of the early Babylonians. The Egyptians developed a baking oven approaching the design of a modern oven about 2700 B.C. Since 1750 B.C. there were professional bakers in Egypt. They were familiar with the raising of wheat doughs with brewer's yeast and even with chemical leavening. In 450 B.C. the Egyptians were familiar with the souring of doughs. But the form of this sour dough bread was still similar to that of a flat bread. Discoveries in tombs have shown that a sour dough bread was known in rye growing areas north of the Alps since the end of the bronze age (800 B.C.). In 100 B.C. the sour dough bread was generally known throughout the world (VON STOKAR, 1951). The Greeks developed the art of baking further, and especially the design of baking ovens. Greek bakers brought the art of baking bread and cake to Rome. The Romans in turn developed the earlier techniques of other populations to a high degree of technical and organizational perfection. They also developed the form of the baking oven which remained the model until the 19th century. In Central Europe the preparation of bread in its proper sense stems from the middle of the first millenium A. C. The further development was very fast, and at the beginning of the 8th century bakers are mentioned in the "lex alamanorum" (ROHRLICH, 1976).

The introduction of the stone oven permitted an increase in the heating surface and an increase in production. The oldest dough kneading machines date from the second half of the 18th century. Finally, the industrial production of baker's yeast in the last century was decisive for the technology of baking. Only this development permitted the production of the loaf bread as we know it today. The concentration of the

Table 1. Wheat and Rye Production of Some Countries in 1977 (in 1000 tons)

Country	Wheat	Rye	Wheat/Rye Ratio
Italy	6330	31	204/1
Bulgaria	3010	20	150/1
Great Britain	5230	38	138/1
USA	55130	432	128/1
Canada	19650	392	50/1
France	17450	376	46/1
Hungary	5310	147	36/1
Argentina	5300	170	31/1
USSR	92040	8470	10.9/1
Czechoslovakia	5240	870	6.0/1
Sweden	1560	364	4.3/1
Austria	1070	351	3.0/1
West Germany	7180	2538	2.8/1
East Germany	3100	1500	2.1/1
Denmark	605	320	1.9/1
Poland	5310	6200	0.9/1
Totals	386600	23770	16 /1

(ROTHE, 1980)

population in towns at the beginning of the 20th century led to technical advances and the rationalization of production. A lack of workers, undesirable working hours, the desire to make the work easier, and to lower production costs contributed to this development. Mainly since 1950 automation has advanced in a broad front and has led to the establishment of some fully automated bakeries. The typical properties of bread, its form, appearance, texture and flavor, were originally produced manually and in conformity with the quality of available grains. Now the baker has to produce bread in the same form and with the same quality by means of rational, technical processes (WASSERMANN, 1981). This change from the manual to the industrial method of producing bread and other baked goods can be observed in almost all industrialized countries. The conditions for this change are particularly favorable in countries in which the assortment of types of breads and baked goods is not very large. Therefore, the development started earlier in countries producing bread almost exclusively from wheat, as in the United States. During the past years the industrial production of rye breads and mixed grain breads has progressed considerably. The production of sweet goods and shelf stable baked goods occurs today already largely in industrial facilities (SEIBEL, 1970).

The development of cereal foods has proceeded through several stages: from roasted grain to gruels to flat breads and finally to leavened bread loaves. But the latter stages have not replaced the earlier ones. Today all stages are still practiced. About 60% of the world population, mainly in Central America, parts of South America, Africa, the Middle East and the Far East, eat gruels made from grain crops and flat breads.

II. The Nutritional Contribution of Bread

The individual grain consists of a starchy endosperm whose outer layers are rich in protein, vitamins, and minerals; a largely indigestible fruit coat (or pericarp); and a germ or embryo. The higher the degree of extraction of the flour, that is, the greater the yield of flour for a given weight of grain, the greater the amount of seed coat and of the outer layer in the flour. Such flours are darker. Short extraction flour (or very white flour) contains almost exclusively portions of the endosperm. Wheat flours generally contain more protein and less fiber than rye flours. The ash content of rye flours is almost twice as high as that of comparable wheat flours.

Grains and products made from milled grains have a favorable combination of nutrients. For 4 billion people they provide more than 50% of the required calories, and in some regions more than 90%. Beyond this they supply considerable percentages of the nutritionally important proteins, B vitamins, and minerals and trace minerals. Bread is a staple food in Germany (as it is in other industrialized countries) and supplies the following percentages of the required nutrients: 20% of the calories, 45–55% of the carbohydrates, 22–25% of the proteins in the form of plant protein. Some specialty breads also contain animal proteins. In addition bread contributes 25–33% of the required vitamins of the B complex (thiamin, riboflavin), 2 grams daily of minerals (among them calcium and iron), as well as a considerable part of the requirement for sodium chloride. If bread is supplemented with foods rich in nutrients such as animal protein, vitamins A, C and D, and some minerals, it can be a suitable basis for a nutritionally optimal diet (CREMER et al., 1969) (Table 2).

Carbohydrates occur mainly in the form of starch (on the average about 45–50% of the weight of fresh bread) (Table 3). Mono- and disaccharides are not very important since they account for less than 5% of the carbohydrates. The carbohydrates of bread serve mainly as carbon and energy sources. In the form of gelatinized starch they are preferable to excessive amounts of carbohydrates in the form of sugar because of the lesser risk of contributing to arteriosclerosis and diabetes (YUDKIN and RODDY, 1964). In addition carbohydrates counteract the tendency toward an excessive intake of fats in industrialized countries. They also aid in the digestion and complete oxidation of fats in humans because of large inner surface and porosity which distributes the fat finely in the stomach and intestinal tract.

Bread, and particularly bread from high extraction flours and whole grain, contains bulk materials. These are the indigestible, organic substances present in the structural molecules and the cell wall of plants. They consist mainly of cellulose, hemicellulose, pectin, and lignin. These substances increase the mass of food in the intestines and increase the feeling of satiety. They regulate the passage of food through the intestinal

Table 2. Contribution of Some Foods to Caloric Supply in West Germany

	Percent of Total Caloric Content 1950	1960	1974
1. Bread flour (75% wheat, 25% rye)	32	26	20
2. Pork	3	5	8
3. Beef	2	3	4
4. Sugar	9	11	13
5. Alcohol	2	5	7
Sum of 2 to 5	16	24	32

(MENDEN et al., 1975)

Table 3. Nutrient Content and Caloric Content of Various Types of Bread

	Moisture %	Crude Fat %	Protein (N·6.25) %	Total Carbohydrates %	Caloric Value per 100 g kJ	Caloric Value per 100 g kcal
Cracked grain rye and whole grain rye bread	43.8	1.2	6.8	45.5	935	220
Rye (flour) bread	42.2	0.9	5.2	47.8	935	220
Rye (mixed grain) bread	41.0	1.4	7.0	49.0	1005	237
Wheat (mixed grain) bread	40.0	1.5	7.5	49.0	1018	240
Wheat (flour) bread	36.2	1.8	8.9	51.6	1104	258
Wheat toast bread	35.0	3.9	8.5	49.9	1141	269
Cracked grain wheat and whole grain wheat bread	43.4	1.2	7.2	44.9	931	219
Knäckebrot	6.4	1.7	11.4	76.2	1554	366

(Rabe and Seibel, 1981)

tract and may have prophylactic and therapeutic functions with regard to gastrointestinal and cardiovascular diseases.

Bread contains only about 5–9% protein and contributes to protein nutrition only in a limited way (Table 3). Nevertheless, the contribution of bread is not negligible. It is the second largest contributor of proteins to the diet after meat protein, and ahead of milk, cheese, and cottage cheese (Seibel and Zentgraf, 1981). The biological value of the protein of grains is low in comparison with proteins of animal origin, at least for the growing organism. Rye bread has a slightly lower protein content than wheat bread but a slightly higher biological value. For flour of 80% extraction it is 75 for rye bread and 65 for wheat bread (Cremer et al., 1969). Prolonged baking lowers the biological value of the protein. This is particularly true if higher concentrations of sugar or sugar containing ingredients are added, as for instance, with milk rolls. The Maillard reaction between the sugar (in this case lactose) and the protein leads to addition compounds which cannot be hydrolyzed by the intestinal enzymes. However, these addition compounds contribute to the aroma of bread.

The limiting amino acid in grain protein is always lysine (2.5% in wheat protein and 3.3% in rye protein), followed by threonine for wheat and presumably by isoleucine and tryptophan for rye (Cremer et al., 1969). The protein of flour of higher extraction and of whole grain has a higher biological value. In countries with a deficiency of animal and vegetable protein sources good results have been obtained with the fortification of wheat flour with synthetic L-lysine and D,L-threonine or with the addition of skimmilk powder. However, the amount of added lysine must be large enough to compensate for the loss of this amino acid through the Maillard reaction (Cremer et al., 1969). The nutritional value of grain proteins is considerably improved by fermentation with sour dough bacteria and with yeast. Yeast contains about 1.3% lysine (as is). Its contribution in straight doughs with 5–7% yeast (based on flour) is considerable (Becker, 1966).

Bread is also important as a source of essential vitamins of the B complex and of vitamin E (Table 4). Normal diets generally contain considerable amounts of riboflavin, niacin, and some other vitamins. However, vitamin B1 occurs in sufficient concentrations only in pork, bread, and a few foods which are not consumed regularly. Therefore, bread is most important for the supply of vitamin B1. Its concentration depends on the degree of extraction of the flour and on the time of baking and the baking tempera-

Table 4. Vitamins in 300 g of Bread

Vitamin	Daily Requirement	Vitamin Content as % of the Recommended Daily Allowance: White Bread	Mixed Bread	Rye Bread	Whole Wheat Bread	Whole Rye Bread	Knäckebrot	Graham-Bread
A	5000 IU	0	0	0	20	–	–	–
E	5 mg	–	–	–	134	–	240	–
B1	1.6 mg	16	25	26	38	28	38	40
B2	1.8 mg	10	17	17	25	23	23	18
Niacin	12 mg	23	37	25	80	42	50	62
B6	1.5 mg	28	–	44	60	–	60	40
Folic acid	0.5 mg	8	–	12	14	–	–	–
Pantothenic acid	6 mg	20	–	–	31	–	–	–

(KRAUT, 1963)

ture. The consumption of light breads covers only about 16% of the daily requirement for vitamin B1 (thiamin). Darker flours and whole grain contain higher concentrations of vitamin B1 as well as niacin, vitamin E, and minerals. Bread which has been baked or roasted for a long time, such as Pumpernickel or Zwieback, shows an almost complete loss of the heat labile vitamin. Yeast contains about 15–30 micrograms of thiamin per gram (as is) and improves the concentration of this vitamin in bread. In several countries in which low extraction flours are used extensively such flours are fortified with a mixture of vitamins (mostly thiamin, riboflavin, and niacin, and occasionally with pyridoxin) and in some countries they are also fortified with calcium and iron (CREMER et al., 1969).

Cereal grains contain only small concentrations of fats but these are not without some importance. The approximately 1.5 to 2.5% of fat in wheat or rye grain is evenly divided between the endosperm and the germ. Linoleic acid and linolenic acid account for 60% of total fats. These fatty acids play a part as carriers of fat soluble vitamins.

The minerals and trace elements of bread are also important (Table 5). Particularly for the darker breads they contribute a considerable percentage of the required elements such as potassium, calcium, iron, magnesium, manganese, and zinc (LUDEWIG, 1975). Rye also contributes fluorine. The major portion of the minerals is found in the outer layers of the grain. Therefore, they are also found in higher concentration in high extraction flours. Wheat flour (type 405) contains 0.4% minerals; cracked wheat contains 1.8%. An average daily intake of 140 g of whole wheat flour provides 46% of the iron, 8% of the calcium and 100% of the zinc requirement of adults. A low extraction flour (type 550) provides only 15% of the iron and 3% of the calcium.

Table 5. Mineral Content in 200 g of Wheat or Rye Whole Meal

Mineral	Daily Requirement (%)
Potassium	60–70
Phosphorus	70–80
Magnesium	70–90
Calcium	10–20
Manganese	30
Iron	50
Copper	50
Zinc	100

(CREMER and ACKER, 1960)

Other bread ingredients such as water, salt, yeast, eggs, dairy products, fruits and dough conditioners enrich the mineral content of bread (LUDEWIG, 1975).

The question of flour fortification has been debated repeatedly. Fortification is