FERMENTATION PRODUCTS

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Contents

Summary

Fermentation was traditionally a process which enabled to preserve food and as such has been used for centuries until present. However nowadays, the main purpose of food fermentation is not to preserve, since other preservation techniques are known, but to produce a wide variety of fermentation products with specific taste, flavor, aroma and texture. Using various microbial strains, fermentation conditions (microorganisms, substrates, temperature, time of fermentation etc.) and chemical engineering achievements, enable us to manufacture hundreds of types of dairy (cheeses, fermented milk products), vegetable (sauerkraut, pickles, olives), meat (fermented sausages) products, breads, alcoholic beverages (wine, beer, cider), vinegar and other food acids, as well as oils. In such a wide variety of products, tastes and textures, surprising is that in the majority of cases, only two types of fermentations are used: lactic acid and ethanolic fermentation. The function of both is to change conditions, so unwanted spoiling or pathogenic microorganisms would not grow and alter the food.

Historically, fermentation products were mainly food products, but in recent years an increased interest has been observed in the production of bulk chemicals (ethanol and other solvents), specialty chemicals (pharmaceuticals, industrial enzymes), biofuels and food additives (flavor modifiers). Fermentation processes are also used in agriculture.

The products are diversified: from traditionally made silages, through single cell protein, ending with microbial pesticides.

1. Introduction

Fermentation products include:

- **food products**: from milk (yogurt, kefir, fresh and ripened cheeses), fruits (wine, vinegar), vegetables (pickles, sauerkraut, soy sauce), meat (fermented sausages: salami)
- **industrial chemicals** (solvents: acetone, butanol, ethanol; enzymes; amino acids)
- **specialty chemicals** (vitamins, pharmaceuticals)

Microbial fermentations can be either homofermentative – single main product, or heterofermentative – mixed products. The main fermentation products include organic acids, ethyl alcohol and carbon dioxide. Commercially the mostly important are lactic acid and ethanolic fermentations. Lactic acid fermentation is used in fermentation of milk, vegetables (cucumber, cabbage, cassava), cereals (wheat, maize), meat and fish. In milk and meat fermentations, starters are necessary, in other – natural flora is sufficient. Alcoholic fermentation is one of the most important and the oldest processes. It is used in the production of alcoholic beverages, chemical and automotive industry, solvents and starting materials for the manufacture of cosmetics and pharmaceuticals, as well as disinfectants in medicine.

1.1. Definition

The term "fermentation" comes from a Latin word *fermentum* (to ferment). The historical definition describes fermentation as the process in which chemical changes in an organic substrate occur as the result of action of microbial enzymes. Fermentation can be described as *respiration without air*. Historically, the science of fermentation is called zymology and the first zymologist was Louis Pasteur, who as the first made yeast responsible for fermentation. Alchemy called fermentation putrefaction – natural rotting or decomposing of substances. Nowadays, it is a metabolic process in which carbohydrates and related compounds are partially oxidized with the release of energy in the absence of any external electron acceptors – organic compounds produced by breakdown of carbohydrates. During fermentation, incomplete oxidation of organic compounds occurs and for this reason less energy is obtained when compared with aerobic oxidation of the compound.

Paradoxically, the term industrial fermentation usually refers to either aerobic or anaerobic processes, whereas fermentation in biochemical context describes a strictly anaerobic process, which occurs if pyruvic acid does not enter the Krebs cycle and if electrons from glucose metabolism do not enter electron transport system. In this process, reduced organic compounds are formed, usually acid by-products. Industrial fermentation, a term used in chemical engineering, describes the process operations that utilize a chemical change induced by a living organism or enzyme, in particular bacteria, yeast, molds or fungi which produce a specified product.

1.2. History

Fermentation of food arose more by accident than by guided efforts. Fermentation has been used since ancient times to conserve and alter foods. For thousand of years fermentation processes were carried out without understanding microbial mechanisms, until XIX century. Fruit fermentation is a natural process and in this context fermentation precedes human history. Greeks even attributed fermentation to one of gods – Dionysos – a god of fruit fermentations. The first fermentations included the production of beer (Babylonia), soy sauce (Japan, China), fermented milk beverages (Balkans and Central Asia) (Table 1). Fermented beverages appeared in 5000 B.C. in Babylon, 3150 B.C. in Ancient Egypt, 2000 B.C. in Mexico and 1500 B.C. in Sudan.

Before World War I the only large-scale fermentation product was ethanol. During World War I, acetone-butanol fermentation was commercially established. Acetone was used in explosives production. Shortly after the war, sharp increase in the market of fermentation products was observed – manufacture of organic acids began. Before World War II fermentation was mainly a method of food production. In the years 1941-46, the market for conventional fermentation products, such as antibiotics, germ warfare, was established. This greatly increased interest in industrial utilization of microorganisms.

Time	Fermentation Product	Place	Ref.
Antiquity	Bread, vinegar, soy sauce, wine, beer		1
7000 B.C.	Beer and wine	Assyria, Caucasia, Mesopotamia, Sumer	2
6000 B.C.	Winemaking	Georgia	3
5000 B.C.	Wine jars	Zagros Mountains, Iran	3
	Fermented beverages	Babylon	3
3000 B.C.	Beer and fermented milk products	Babylon	3, 4
2600 B.C.	Bread	Egypt	3, 5
1000 B.C.	Say sauce and miso	China	5
600 B.C.	Cheese	Asia	5
500 B.C.	Preservation of fish and meat		
100 B.C.	Bread	Ancient Rome	2
Modern times			
1700’s	Vinegar – from fruit pomace		5
	Gallic acid		5
1800’s	Yeast induce fermentation	Erxleben, Germany	1
1850’s	1) Bacteria produce lactic acid which conserves food 2) Pasteurization – heat treatment to prevent unwanted fermentation 3) Yeast+grape juice $\rightarrow$ wine – beginning of the science of food fermentation	Louis Pasteur, France	1, 6
end of 1800’s	Composting		5

1900's	Aseptic fermentation (exclusion of unwanted microorganisms)		1
New fermentations			
	Industrial production of acetone, butanol, butanodiol – substrates for rubber production		1
1930	Discovery of biotechnological method of organic acids production – citric and gluconic acid		1, 5
1940's	Industrial production of organic acids		1
1950's	Industrial penicillin production – first high-cost and high-tech process. First large-scale production of pharmaceuticals. Penicillin was the first antibiotic – a medication against bacterial infections		1, 4, 5
1950's-60's	Steroid transformations by fungal spores		5
1960's	Commercial production of amino acids by fermentation; Production of MSG (monosodium glutamate, flavor enhancer)	Japan	1
1960's-80's	Mycotoxins, treatment and reuse of wastes (animal, plant and domestic)		5
1970's	Microbiologically produced enzymes – used in grain processing, sugar production, fruit juice clarification, detergent additives		1
1980's	Genetic engineering techniques – insulin production		1

1 Mirbach and El Ali, 2005
2 Pretorius et al., 2003
3 Steinkraus, 1995
4 Nielsen, 1999
5 Zheng and Shetty, 2000
6 Fung, 2000; Teuber, 2000

Table 1. History of fermentation products

1.3. Theory

Fermentation processes can be classified as spontaneous and induced (e.g. making bread dough by the addition of baking yeast to flour). Fermentation products contain chemical energy, which means that are not fully oxidized and their complete mineralization requires oxygen. Fermentation is less energetically efficient than oxidative phosphorylation (ATP is produced only by substrate-level phosphorylation). While fermentation of 1 molecule of glucose yields 2 molecules of ATP, in aerobic respiration 36 ATP molecules are formed. The final step of fermentation, transformation of pyruvate into end products, does not generate the energy, but produces NAD^+ that is required for glycolysis.

1.4. Benefits and Pitfalls of Fermentation

Benefits of fermentation include conversion of sugars and other carbohydrates: juice into wine, grain to beer or CO_2 to leaven bread, sugars in vegetables to preservative organic acids. Fermentation extends shelf life of foods, adds aroma and flavor, in some cases increases the content of vitamins and improves digestibility, comparing with raw materials. It can also reduce or increase toxicity. Table 2 discusses benefits and pitfalls of fermentations.

Benefits/Pitfalls	**Description**
BENEFITS	
General advantages	– mild conditions (pH and temperature) – development of unique flavors and textures of food – low consumption of energy – low capital and operating costs – relatively simple technologies.
Pathogenic bacteria and spoilage organisms are inhibited	The most food is fermented by lactic acid fermentation, during which pH is lowered to ca. 4. Also, bacteriocins, hydrogen peroxide, ethanol, diacetyl are produced. This inhibits the growth of unwanted microorganisms and prevents spoilage of food.
Detoxification and softening	Lactic acid fermentation also may reduce the content of natural toxins in plant food: e.g. cyanogenic glycosides in cassava (major staple food in Africa) and soften plant tissues.
Enhanced digestibility – degradation of oligosaccharides and dietary fiber	Complementary foods for children containing amylase-rich flour and lactic acid bacteria. Also, fermentation of plant foods favors transformation of phytate by phytase. This increases several fold bioavailability of iron. The consequence of lactic acid fermentation is decreased tannin content in cereals, which increases minerals absorption and protein digestibility of grains.
Beneficial health effects	Fermentation improves food safety and quality through the presence of probiotics that protect from *E. coli* and other pathogens and have hypocholesterolemic and anticarcinogenic effects, which is of particular significance in lactose intolerance and gastrointestinal disorders.
PITFALLS	
Fermentation technologies are complex and sensitive and require careful control of:	– quality and safety of raw materials – initial level of contamination – environmental hygiene and sanitation – safety of metabolites – processing conditions and degree of acidity achieved
Risk of contamination	If the fermentation was not properly conducted, spoilage may appear which causes annoying odor, bad taste (butyric acid, hydrogen sulfide, aromatic amines). Also, there is a danger of contamination by pathogenic bacteria
Risk of intoxication	There were reported cases of dangers associated with the consumption of fermented food. In Alaska, fish, seafood and birds were traditionally fermented in grass-lined hole. In 1980's the fermentation began to be carried out in plastic containers. This resulted in the development of botulinum bacteria which thrive under anaerobic conditions and caused several botulism cases

Table 2. Benefits and pitfalls of fermentation.
[Bekers et al., 1997; Beumer, 2001; Fellows, 2000; Mirbach and El Ali, 2005; Motarjemi et al., 2001].

1.5. Effect on Foods

Fermentation of foods is the controlled action of microorganisms to alter the texture of food, to preserve (by the production of acids and alcohols) and to produce characteristic flavors and aromas. Changes produced by fermentation in food are discussed in Table 3.

Change	Description
Texture	food is softened as the result of complex changes in proteins and carbohydrates
Nutritional value	microorganisms improve digestibility by hydrolysis of polymeric compounds, mainly polysaccharides and proteins; secrete e.g. vitamins
Enrichment with	protein, essential amino acids, essential fatty acids
Flavor	sugars are fermented to acids, which reduce sweetness and increase acidity, in some cases bitterness is reduced by enzymatic activity
Aroma	the production of volatile compounds: amines, fatty acids, aldehydes, esters and ketones
Color	proteolytic activity, degradation of chlorophyll and enzymatic browning may produce brown pigments

Table 3. Changes produced by fermentation in food.
[Batty and Folkman, 1983; Fellows, 2000; Whitaker et al., 1997]

2. Fermentation Feedstocks

2.1. Microorganisms

Microorganisms that are used in industrial fermentations include:

Bacteria: *Acetobacter, Streptococcus, Lactococcus, Leuconostoc, Pediococcus, Lactobacillus, Propionibacterium, Brevibacterium, Bacillus, Micrococcus, Staphylococcus.*

Yeast: *Saccharomyces, Candida, Torulopsis, Hansenula*

Mold: *Aspergillus, Penicillium, Rhizopus, Mucor, Monascus, Actinomucor.*

Lactic acid bacteria (LAB) are naturally present in milk, fruit juice, plant products, intestine and mucosa. In fermentation products, antimicrobial effect of their acids is used. Lactic acid bacteria are divided into three groups:

- homolactic (*Streptococcus* spp., *Pediococcus* spp.)
- heterolactic (*Leuconostoc* spp.)
- facultative (*Lactobacillus* spp.).

Generally, *Lactobacilli* are stronger acid producers than *Streptococci.*

Most LAB produce bacteriocins, which reduce the use of chemical preservatives, e.g. *Lactococcus lactis* produces nisin which inhibits growth of *Cl. botulinum* and *Listeria monocytogenes*). Some LAB have stabilizing and viscosity forming properties. This enables us to avoid using synthetic stabilizers and emulsifiers. Yeasts are frequently minority companions of LAB and are also used to produce CO_2 (in beer and breadmaking) and ethanol (alcoholic beverages). Molds are used in the production of enzymes which degrade polymeric components: cell wall polysaccharides, proteins, lipids, which is significant for texture, flavor and nutritional value of mold fermented foods.

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Biographical Sketch

Katarzyna Chojnacka graduated from Department of Chemistry of Wrocław University of Technology in 1999. She obtained MSc in the field of Biotechnology, specialization Environmental Chemistry. The topic of MSc thesis was wastewater treatment processes by blue-green algae *Spirulina* sp. After graduation, she began Ph.D. studies at Institute of Chemical Engineering and Heating Equipment, at Wrocław University of Technology. During the study she was granted with Socrates/Erasmus scholarship

at University of Calabria, Italy and worked under the guidance of Professor Enrico Drioli and Dr. Lidietta Giorna in the field of lactic acid production in the membrane fermenter. In 2001, she was awarded by European Membrane Society for the work “Using microfiltration to assist in processes of biosorption and bioaccumulation of heavy metal ions in microalgae”.
In 2003 she defended her Ph.D. thesis on using biosorption and bioaccumulation processes in the removal of heavy metal ions from wastewater by a microalga *Spirulina* sp. and became a lecturer at Wrocław University of Technology.

Since 2004 she was appointed to the position of Associate Professor at Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology. She continued research work on biosorption and bioaccumulation, in particular on various applications of these processes - elaboration and development of technologies and techniques which employ these processes. The applications include elaboration of technology of biological feed supplements with microelements for livestock, in which the biomass of algae and aquatic plants is enriched with these constituents by biosorption and bioaccumulation. She has also worked in the field of the application of low-cost biosorbents – materials of plant and animal origin in wastewater treatment, as well as on other applications of bioaccumulation – biomonitoring and elaboration of techniques which reduce bioaccumulation from the polluted environment. She is currently supervising MSc works and co-supervising Ph.D. theses in this field. In 2008 she defended Dr.Sc. (habilitation) thesis “Studies on the application of biosorption and bioaccumulation processes”.

She has published over 70 papers, including 30 original papers in world-known scientific journals and 10 papers in Polish journals and also contributed to the book “Hazardous Materials and Wastewater: Treatment, Removal and Analysis” by Nova Science Publishers: Ch. 10 “The Application of Biosorption and Bioaccumulation of Toxic Metals in Environmental Pollution Control” (by K. Chojnacka). She participated in international and national conferences as invited speaker. She was awarded many times by Polish Minister of Education and also by President of Wrocław University of Technology and Dean of Department of Chemistry. She was awarded by Master of Technology title of Lower Silesia and also by Polish Chamber of Chemical Industry.