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Food Preservation by Fermentation and Fermented food products

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ABSTRACT

Fermentation is an essential metabolic phenomenon that basically takes place in absence of oxygen (O2). In the process of fermentation, sugar is consumed in the absence of oxygen. The products formed due to fermentation are organic acids, gases, or alcohol. Fermentation occurs commonly in yeast and bacteria and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. In terms of microbiologists, fermentation is a primary means of producing ATP by the degradation of organic nutrients anaerobically, in presence of suitable microorganisms. Zymology is the science of fermentation. Fermentation processes are believed to have been developed in order to preserve fruits and vegetables for times of scarcity by preserving the food by organic acid and alcohols, impart desirable flavour, texture to foods, reduce toxicity and decrease cooking time. Fermentation is a desirable process and the products that are being prepared by natural fermentation are fermented products. That includes pickles, sauerkraut, yogurt, kimchi, cheese, etc

Key words: Fermentation, fermented products, probiotics, healthy food, nutraceutical

INTRODUCTION:

Fermentation is an essential metabolic phenomenon that basically takes place in absence of oxygen (O_2). In the process of fermentation, sugar is consumed in the absence of oxygen. The products formed due to fermentation are organic acids, gases, or alcohol. Fermentation occurs commonly in yeast and bacteria and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. In terms of microbiologists, fermentation is a primary means of producing ATP by the degradation of organic nutrients anaerobically, in presence of suitable microorganisms. Zymology is the science of fermentation. Fermentation processes are believed to have been developed in order to preserve fruits and vegetables for times of scarcity by preserving the food by organic acid and alcohols, impart desirable flavour, texture to foods, reduce toxicity and decrease cooking time [1]. When the fermentation term is used in case of fruits and vegetables, it is known as pickling. Fermented fruits and vegetables have an important role in feeding the world's population [2,3]. It is well documented that fermented drinks were consumed in Babylon 5000 years ago [4] and that bread was consumed in Egypt around the 1500 BC [5]. Fermentation is employed in the production of foods through the application of microorganisms or their enzymes [6]. Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases; lipases hydrolyze the polysaccharides, proteins and lipids to nontoxic products with flavors, aromas and textures pleasant and attractive to the human consumer [7]. Humans have used fermentation to produce drinks and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation occurs within the gastrointestinal tracts of all ruminant animals, including humans.

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Brief history about fermentation

Preservation by fermentation begins with the old days when the sailors travelling long distance used to carry fermented fruits and vegetable with them so as to ensure long term preservation. Since man knew the art of harnessing the fermentation technology, French chemist Louis Pasteur was the first *zymologist*, when in 1857 he connected yeast to fermentation. Pasteur originally defined fermentation as respiration without air. Louis Pasteur was one of the pioneer researcher on food preservation who proclaimed that the microorganisms that are developed from tiny inoculums are not spontaneously generated. Pasteur performed careful research and concluded, *"I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development and multiplication of cells.... If asked, in what consists the chemical act whereby the sugar is decomposed ... I am completely ignorant of it."* The German Eduard Buchner, winner of the 1907 Nobel Prize in chemistry, later determined that fermentation was actually caused by a yeast secretion that he termed *zymase*. The research efforts undertaken by the Danish Carlsberg scientists greatly accelerated the gain of knowledge about yeast and brewing. The Carlsberg scientists are generally acknowledged with jump-starting the entire field of molecular biology.

BIOCHEMISTRY OF FERMENTATION

Fermentation is essentially takes place in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (Adenosine triphosphate) by glycolysis. During fermentation pyruvate is metabolized to diverse compounds.

3 types of fermentation usually take place:

a. **Homo-lactic** fermentation is the production of lactic acid from pyruvate.
b. **Hetero-lactic** fermentation is the production of lactic acid as well as other acids and alcohols.
c. **Alcoholic** fermentation is the conversion of pyruvate into ethanol and carbon dioxide.

Some typical examples of fermented products are **ethanol**, **lactic acid**, and **hydrogen**. Several other exotic compounds can be produced by fermentation that includes butyric acid and acetone.

The final step of fermentation i,e. conversion of pyruvate to fermentation end-products does not produce any energy. This is critical for an anaerobic cell since it rejuvenates **nicotinamide adenine dinucleotide (NAD)**, which is required for the glycolysis process. This is having imperative role for normal cellular function, as glycolysis is the only source of ATP in anaerobic conditions

Fermentation turns NADH and pyruvate produced in glycolysis into NAD^+ and an organic product. In the presence of O_2, NADH and pyruvate are used to generate ATP in the process of respiration. This process is known as oxidative phosphorylation. It generates much more ATP as compared to glycolysis alone. The magical process of fermentation is rarely utilized when oxygen is available. The process is necessarily anaerobic which means that it only takes place in absence of oxygen (O_2). Obligate anaerobes are those anaerobes which cannot tolerate oxygen at even physiological range of concentration.

The first step is the Embden – Meyerof - Parnas glycolysis which is same in most of the fermentation pathways:

$$\mathbf{C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow 2\ CH_3COCO_2^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+}$$

Pyruvate is $CH_3COCO_2^-$. Pi is inorganic phosphate. Two ADP molecules and two Pi are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD^+ are also reduced to NADH.

In oxidative phosphorylation, the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via an electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction).

The reaction differs according to the sugar being used in the process of fermentation, as well as the particular organism performing it. Below, the sugar is glucose ($C_6H_{12}O_6$), the most common sugar and the process is the alcoholic fermentation.

Chemical Equation

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 2ATP$ (Released energy: 118 kJ mol^{-1})

Word Equation

Sugar (glucose) → Alcohol (ethanol) + Carbon Dioxide (CO_2) + Energy (as ATP)

TYPES OF FERMENTATION

1. Ethanol fermentation

Ethanol fermentation is the process in which glucose is converted to **ethanol (C_2H_5OH)** and **carbondioxide (CO_2).** Ethanol fermentation is also known as alcoholic fermentation that converts sugars such as glucose, fructose, and sucrose into energy (ATP), ethanol and carbon dioxide as by-products. One glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules. yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process.

$C_6H_{12}O_6 \rightarrow$ Pyruvate $\rightarrow 2C_2H_5OH + 2\ CO_2$

Ethanol fermentation / yeast fermentation have wide practical applications in the production of **alcoholic beverages, ethanol** and **bread.** This process is mainly responsible for the existence of any sort of alcohol. Man have harnessed the potential of controlled bio-fermentation to achieve huge potential **wine industry, bread industry** and **cheese industry**

Basically the reaction takes place in 3 steps:

Step 1: Enzyme **invertase** cleaves the glycosidic linkage in disaccharide sucrose between the glucose and fructose molecules in.

$C_{12}H_{22}O_{11} + H_2O +$ invertase $\rightarrow 2C_6H_{12}O_6$

Step 2: Glucose molecule is broken down into two **pyruvate** molecules (glycolysis).

$C_6H_{12}O_6 + 2ADP + 2Pi + 2NAD^+ \rightarrow 2CH_3COCOO^- + 2ATP + 2NADH + 2H_2O + 2H^+$

Step 3: pyruvate is converted to **ethanol** and **CO_2** (by two step reaction)

$CH_3COCOO^- + H^+ \rightarrow CH_3CHO + NADH + H^+ \rightarrow C_2H_5OH + NAD^+$ (in presence of pyruvate decarboxylase and alcohol dehydrogenase)

2. Lactic Acid Bacteria Fermentation

Out of the various approaches to fermentation, lactic acid fermentation is strictly controlled by salt concentration. Lactic acid bacteria fermentation is done by using natural microflora or lactic acid bacterial (LAB) cultures which is employed throughout the world, in conjunction with chemical preservation, using salt and acid to preserve various foods such as milk, cereals, meat, and fruits and vegetables [8]. Lactic acid (LA) fermentation of vegetables and fruits is a common practice to maintain and improve the nutritional and sensory features of food commodities Lactic acid fermentation retains all the natural plant ingredients while improving the quality, taste and

aroma [9]. LA fermentation enhances the organoleptic and nutritional quality of the fermented fruits and vegetables and retains the nutrients and coloured pigments [10]. Fermentation plays at least five roles in food processing:

i. Enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food.
ii. Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid, alkaline fermentations and high salt fermentations;
iii. Enrichment of food substrates biologically with vitamins, protein, essential amino acids and essential fatty acids;
iv. Detoxification during food fermentation processing.
v. Decrease in cooking times and fuel requirements [11, 12]

Lactic acid fermentation is used in many areas of the world to produce foods that cannot be produced through other methods. The most commercially important genus of lactic acid-fermenting bacteria is *Lactobacillus*, though other bacteria and even yeast are sometimes used. Two of the most common applications of lactic acid fermentation are in the production of yogurt and sauerkraut. Lactic acid is naturally produced in fermented products from the sugar present in the fruit sample. Lactic acid bacteria is a group of gram positive, non-spore forming, cocci or rod which produce lactic acid as a major end product. The basic mechanism of the preservation of foods is the production of acid, chiefly by LAB, which lowers the pH to a level at which most of the spoilage-causing microorganisms cannot grow, and, thus, the food is preserved [13,14]. Food substrates overgrown with desirable, edible microorganisms become resistant to invasion by spoilage, toxic or food poisoning microorganisms. Other, less desirable or pathogenic organisms find it difficult to compete [15,16]. The consumption of LA fermented fruits and vegetables helps to enhance human nutrition in several ways such as the attainment of balanced nutrition, providing vitamins, minerals, and carbohydrates, and preventing several diseases such as diarrhoea and cirrhosis of liver because of probiotic properties [17]. Probiotic is a relatively new word meaning “for life” and it is generally used to name the bacteria associated with beneficial effects for humans [18,19]. Probiotics are defined as live microbial feed such as *Lactobacillus plantarum*, *L. casei*, *L. acidophilus*, and *Streptococcus lactis* which are supplemented by food that beneficially affect the host by improving its intestinal balance [20]. Several studies have shown that supplementation of probiotics to food provides several health benefits such as reduction of serum cholesterol, improved gastrointestinal function, enhanced immune system, and lower risk of colon cancer [21,22,23,24]. A number of studies have found probiotics consumption to be useful in the treatment of diarrhea, lactose intolerance, colon cancer, cholesterol, blood pressure, immune function and infections, mineral absorption, irritable bowel syndrome and colitis. Important probiotic bacteria can be listed as *Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Bifidobacterium longum, Pediococcus acidilactici* and *Saccharomyces boulardii* [25]. The genus *Lactobacillus* is a heterogeneous group of LAB with important application in food and feed fermentation. *Lactobacilli* are used as probiotics inoculants and as starters in fermented food [26]. The genus *Lactobacillus* is Gram-positive organisms which produce lactic acid by fermentation which belongs to the large group of LAB. Other genera such as *Lactococcus*, *Enterococcus, Oenococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc,* and *Lactobacillus* are also considered in LAB group due to lactic acid production ability [27].

The LAB tolerate high salt concentrations, which give them an advantage over other less salt-tolerant species and allows the LAB to produce acid that inhibits the growth of undesirable microorganisms [28]. *Leuconostoc* sp. is also known for its high salt tolerance, and, for this reason, initiates the majority of lactic acid fermentations [29]. The addition of salt to the pickles restricts the growth of gram-negative bacteria and enhances the growth of LAB and *Leuconostoc* sp. LAB are one of the important microorganisms in food fermentation, and have been shown by serological techniques and 16'S ribosomal RNA cataloging to be phylogenetically related and to share a number of common features [30]. Lactic acid fermentation increases shelf life of fruits and vegetables and also enhances several beneficial properties, including nutritive value and flavour, and reduces toxicity. LAB are recognized for their fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits [31,32,33,34]. Fermented fruits and vegetables can be used as a potential source of probiotics as they harbor several lactic acid bacteria such as *Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Pediocccus cerevisiae, Streptococcus thermophilus, Streptococcus lactis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus citrovorum, Bifidobacterium bifidus* and *L. pallax* while *Staphylococcus aureus*, *Saccharomyces cerevisiae*, and *A. niger* may be present when a pickle is spoiled. Spoilage of pickles can also be due to microbial contamination or oxidation rancidity of the oil used.

Some products made by harnessing the principle of Lactic Acid Bacteria fermentation includes:

A. **Pickle** – It's a product prepared by lactic acid bacteria (LAB) fermentation of sugar present in pieces of fruits and vegetables. The prepared product is rich in Lactic acid and only the beneficial bacteria that can tolerate lactic acid pH survive.

B. **Sauerkraut** – It is basically a finely cut cabbage that has been fermented by various lactic acid bacteria. Sauerkraut usually has a long shelf life and a distinctive sour flavor.

C. **Yogurt** – Yogurt is basically a fermented product prepared from milk. The main method of producing yogurt is through the lactic acid fermentation of milk with harmless bacteria. The primary bacteria used are typically *Lactobacillus bulgaricus* and *Streptococcus thermophiles*.

D. **Kimchi** – It is basically a Korean dish. It is a staple food in Korean cuisine, is a traditional side dish made from salted and fermented vegetables, most commonly napa cabbage and Korean radishes, with a variety of seasonings including chili powder, scallions, garlic, ginger, and jeotgal.

3. Acetic Acid Bacteria fermentation: AAB are a group of gram-negative bacteria which **oxidize sugars or ethanol** and **produce acetic acid** during fermentation. The acetic acid bacteria consist of 10 genera in the family *Acetobacteraceae*. Several species of acetic acid bacteria are used in industry for production of certain foods and chemicals. Vinegar is formed when acetic acid bacteria is added to alcoholic beverages. In this process, **oxidative fermentation** takes place that creates **vinegar** as a by-product. This process is somewhat aerobic. Weakly fermented liquors very often become sour on exposure to the air. This is owing to the conversion of the alcohol in acetic acid. Acetic acid is produced by fermenting various substrates (starchy solution, sugar solutions or alcoholic foodstuffs such as wine or cider) in presence of Acetobacter bacteria. *Acetobacter aceti* is usually used to produce vinegar (14 per cent acetic acid).

Starchy solution/ Sugar solutions/ Alcohol + O_2 → CH_3COOH

Different Fermented Products

Fermented products are of immense importance to mankind since time immemorial. Fermented products are not only a source of probiotics but also have good palatable qualities. Various products produced by the process of fermentation are actually waste products produced during the reduction of pyruvate to regenerate NAD^+ in the absence of oxygen. Bacteria generally produce acids by fermentation process. Vinegar (5% acetic acid) is the direct result of bacterial metabolism. In the mechanism of vinegar production, Bacteria convert the alcohol to acetic acid. In milk, the acid coagulates the casein to produce curds. In pickling, due to the presence of salt only the lactic acid bacteria survive. The LAB actually converts the sugar present in the pickling material and produce lactic acid. The acid thus produced, preserves the food from pathogenic and putrefactive bacteria. When yeast fermentation takes place it breaks down the glucose ($C_6H_{12}O_6$) into exactly two molecules of ethanol ($C_2H_6O_5$) and two molecules of carbon dioxide (CO_2). Ethanol fermentation breaks the pyruvate down into ethanol and carbon dioxide. This is a mandatory process in bread-making, brewing, and wine-making. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced. Usually only one of the products is desired; in bread the alcohol is baked out, and in alcohol production the carbon dioxide is released into the atmosphere. Lactic acid fermentation breaks down the pyruvate into lactic acid in presence of Lactic Acid bacteria. It takes place in the muscles of animals when they need energy faster than the blood can supply oxygen. It generally takes place during vigorous exercise. It also takes place in bacteria and fungi. It is this type of bacteria that convert lactose into lactic acid in yogurt, giving it its sour taste.

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