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Microorganism-Produced Enzymes in the Food Industry

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1. Introduction

The application of microorganisms, such as bacteria, yeasts and principally fungi, by the food industry has led to a highly diversified food industry with relevant economical assets. Fermentation, with special reference to the production of alcoholic beverages, ethyl alcohol, dairy products, organic acids and drugs which also comprise antibiotics are the most important examples of microbiological processes.

The enzyme industry, as it is currently known, is the result of a rapid development of biotechnology, especially during the past four decades. Since ancient times, enzymes found in nature have been used in the production of food products such as cheese, beer, wine and vinegar (Kirk et al., 2002).

Enzymes which decompose complex molecules into smaller units, such as carbohydrates into sugars, are natural substances involved in all biochemical processes. Due to the enzymes' specificities, each substratum has a corresponding enzyme.

Although plants, fungi, bacteria and yeasts produce most enzymes, microbial sources-produced enzymes are more advantageous than their equivalents from animal or vegetable sources. The advantages assets comprise lower production costs, possibility of large-scale production in industrial fermentors, wide range of physical and chemical characteristics, possibility of genetic manipulation, absence of effects brought about by seasonality, rapid culture development and the use of non-burdensome methods. The above characteristics make microbial enzymes suitable biocatalysts for various industrial applications (Hasan et al., 2006).

Therefore, the identification and the dissemination of new microbial sources, mainly those which are non-toxic to humans, are of high strategic interest. Besides guaranteeing enzyme supply to different industrial processes, the development of new enzymatic systems which cannot be obtained from plants or animals is made possible and important progress in the food industry may be achieved.

2. Fungus of industrial interest

Owing to progress in the knowledge of enzymes, fungi acquired great importance in several industries since they may improve various aspects of the final product.

In fact, the fungi kingdom has approximately 200 species of *Aspergillus* which produce enzymes. They are isolated from soil, decomposing plants and air. *Aspergillus* actually

produces a great number of extracellular enzymes, many of which are applied in biotechnology. *Aspergillus flavus*, *A. niger, A. oryzae, A. nidulans, A. fumigatus, A. clavatus, A. glaucus, A. ustus* and *A. versicolor* are the best known.
The remarkable interest in *Aspergillus niger*, a species of great commercial interest with a highly promising future and already widely applied in modern biotechnology, is due to its several and diverse reactions (Andersen et al, 2008).
Moreover, *A. niger* not only produces various enzymes but it is one of the few species of the fungus kingdom classified as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA). The species is used in the production of enzymes, its cell mass is used as a component in animal feed and its fermentation produces organic acids and other compounds of high economic value (Couto and Sanroman, 2006; Mulimania and Shankar, 2007).

2.1 Microbial enzymes for industries

2.1.1 Pectinase enzyme

Plants, filamentous fungi, bacteria and yeasts produce the pectinase enzymes group with wide use in the food and beverages industries. The enzyme is employed in the food industries for fruit ripening, viscosity clarification and reduction of fruit juices, preliminary treatment of grape juice for wine industries, extraction of tomato pulp (Adams et al., 2005), tea and chocolate fermentation (Almeida et al. 2005; da Silva et al., 2005), vegetal wastes treatment, fiber degumming in the textile and paper industries (Sorensen, et al. 2004; Kaur, et al. 2004, Taragano, et al., 1999, Lima, et al., 2000), animal nutrition, protein enrichment of baby food and oil extraction (Da Silva et al., 2005, Lima, et al., 2000).
The main application of the above mentioned enzyme group lies within the juice processing industry during the extraction, clarification and concentration stages (Martin, 2007). The enzymes are also used to reduce excessive bitterness in citrus peel, restore flavor lost during drying and improve the stableness of processed peaches and pickles. Pectinase and β-glucosidase infusion enhances the scent and volatile substances of fruits and vegetables, increases the amount of antioxidants in extra virgin olive oil and reduces rancidity.
The advantages of pectinase in juices include, for example, the clarification of juices, concentrated products, pulps and purees; a decrease in total time in their extraction; improvement in the production of juices and stable concentrated products and reduction in waste pulp; decrease of production costs; and the possibility of processing different types of fruit (Uenojo and Pastore 2007). For instance, in the production of passion fruit juice, the enzymes are added prior to filtration when the plant structure's enzymatic hydrolysis occurs. This results in the degradation of suspended solids and in viscosity decrease, speeding up the entire process (Paula, et al., 2004).
Several species of microorganisms such as *Bacillus, Erwinia, Kluyveromyces, Aspergillus, Rhizopus, Trichoderma, Pseudomonas, Penicillium* and *Fusarium* are good producers of pectinases (De Gregorio, et al., 2002). Among the microorganisms which synthesize pectinolytic enzymes, fungi, especially filamentous fungi, such as *Aspergillus niger* and *Aspergillus carbonarius* and *Lentinus edodes*, are preferred in industries since approximately 90% of produced enzymes may be secreted into the culture medium (Blandino et al., 2001).
In fact, several studies have been undertaken to isolate, select, produce and characterize these specific enzymes so that pectinolytic enzymes could be employed not only in food processing but also in industrial ones. High resolution techniques such as crystallography

and nuclear resonance have been used for a better understanding of regulatory secretion mechanisms of these enzymes and their catalytic activity. The biotechnological importance of microorganisms and their enzymes triggers a great interest toward the understanding of gene regulation and expression of extracellular enzymes.

2.1.2 Lipases

Lipolytic enzymes such as lipases and esterases are an important group of enzymes associated with the metabolism of lipid degradation. Lipase-producing microorganisms such as *Penicillium restrictum* may be found in soil and various oil residues. The industries Novozymes, Amano and Gist Brocades already employ microbial lipases.

Several microorganisms, such as *Candida rugosa, Candida antarctica, Pseudomonas alcaligenes, Pseudomonas mendocina* and *Burkholderia cepacia*, are lipase producers (Jaeger and Reetz, 1998). Other research works have also included *Geotrichum* sp. (Burkert et al., 2004), *Geotrichum candidum* DBM 4013 (Zarevúcka et al., 2005), *Pseudomonas cepacia, Bacillus stearothermophilus, Burkholderia cepacia* (Bradoo et al., 2002), *Candida lipolytica* (Tan et al., 2003) *Bacillus coagulans* (Alkan et al., 2007), *Bacillus coagulans* BTS-3 (Kumar et al., 2005), *Pseudomonas aeruginosa* PseA (Mahanta et al., 2008), *Clostridium thermocellum* 27405 (Chinn et al., 2008), *Yarrowia lipolytica* (Dominguez et al., 2003) and *Yarrowia lipolytica* CL180 (Kim et al., 2007).

The fungi of the genera *Rhizopus*, *Geotrichum*, *Rhizomucor*, *Aspergillus*, *Candida* and *Penicillium* have been reported to be producers of several commercially used lipases.

The industrial demand for new lipase sources with different enzymatic characteristics and produced at low costs has motivated the isolation and selection of new lipolytic microorganisms. However, the production process may modify their gene expression and change their phenotypes, including growth, production of secondary metabolites and enzymes. Posterior to primary selection, the production of the enzyme should be evaluated during the growth of the promising strain in fermentation, in liquid medium and / or in the solid state (Colen et al., 2006). However, it is evident that each system will result in different proteins featuring specific characteristics with regard to reactions' catalysis and, consequently, to the products produced (Asther et al., 2002).

2.1.3 Lactase

Popularly known as lactase, beta-galactosidases are enzymes classified as hydrolases. They catalyze the terminal residue of b-lactose galactopiranosil (Galb1 - 4Glc) and produce glucose and galactose (Carminatti, 2001). Lactase's production sources are peaches, almonds and certain species of wild roses; animal organisms, such as the intestine, the brain and skin tissues; yeasts, such as *Kluyveromyces lactis*, *K. fragilis* and *Candida pseudotropicalis*; bacteria, such as *Escherichia coli*, *Lactobacillus bulgaricus*, *Streptococcus lactis* and *Bacillus* sp; and fungi, such as *Aspergillus foetidus*, *A. niger*, *A. oryzae* and *A. Phoenecia*.

The b-galactosidase may be found in nature, or rather, in plants, particularly almonds, peaches, apricots, apples, animal organs such as the intestine, the brain, placenta and the testis.

Lactase is produced by a widely diverse fungus population and by a large amount of microorganisms such as filamentous fungus, bacteria and yeast (Holsinger, 1997; Almeida and Pastore, 2001).

Beta-galactosidase is highly important in the dairy industry, in the hydrolysis of lactose into glucose and galactose with an improvement in the solubility and digestibility of milk and dairy products. Food with low lactose contents, ideal for lactose-intolerant consumers, is thus obtained (Mahoney, 1997; Kardel et al. 1995; Pivarnik et al., 1995). It also favors consumers who are less tolerant to dairy products' crystallization, such as milk candy, condensed milk, frozen concentrated milk, yoghurt and ice cream mixtures, (Mahoney, 1998; Kardel et al., 1995). It also produces oligosaccharides (Almeida and Pastore, 2001), the best biodegradability of whey second to lactose hydrolysis (Mlichová; Rosenberg, 2006).

2.1.4 Cellulases

Cellulases are enzymes that break the glucosidic bonds of cellulose microfibrils, releasing oligosaccharides, cellobiose and glucose (Dillon, 2004). These hydrolytic enzymes are not only used in food, drug, cosmetics, detergent and textile industries, but also in wood pulp and paper industry, in waste management and in the medical-pharmaceutical industry (Bhat and Bhat, 1997).

In the food industry, cellulases are employed in the extraction of components from green tea, soy protein, essential oils, aromatic products and sweet potato starch. Coupled to hemicellulases and pectinases they are used in the extraction and clarification of fruit juices. After fruit crushing, the enzymes are used to increase liquefaction through the degradation of the solid phase.

The above enzymes are also employed in the production process of orange vinegar and agar and in the extraction and clarification of citrus fruit juices (Orberg 1981). Cellulases supplement pectinases in juice and wine industries as extraction, clarification and filtration aids, with an increase in yield, flavor and the durability of filters and finishers (Pretel, 1997).

Cellulase is produced by a vast and diverse fungus population, such as the genera *Trichoderma*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Fusarium* and *Phoma*; aerobic bacteria, such as *Acidothermus*, *Bacillus*, *Celvibrio*, *pseudonoma*, *Staphylococcus*, *Streptomyces* and *Xanthomonas*; and anaerobic bacteria, such as *Acetovibrio*, *Bacteroides*, *Butyrivibrio*, *Caldocellum*, *Clostridium*, *Erwinia*, *Eubacterium*, *Pseudonocardia*, *Ruminococcus* and *Thermoanaerobacter* (Moreira & Siqueira, 2006; Zhang et al., 2006). *Aspergillus* filamentous fungi stand out as major producers of cellulolytic enzymes. It is worth underscoring the filamentous fungus *Aspergillus niger*, a fermenting microorganism, which has been to produce of cellulolytic enzymes, organic acids and other products with high added value by solid-state fermentation processes. (Castro, 2006, Chandra et. al., 2007, Castro & Pereira Jr. 2010)

2.1.5 Amylases

Amylases started to be produced during the last century due to their great industrial importance. In fact, they are the most important industrial enzymes with high biotechnological relevance. Their use ranges from textiles, beer, liquor, bakery, infant feeding cereals, starch liquefaction-saccharification and animal feed industries to the chemical and pharmaceutical ones.

Currently, large quantities of microbial amylases are commercially available and are almost entirely applied in starch hydrolysis in the starch-processing industries.

The species *Aspergillus* and *Rhizopus* are highly important among the filamentous fungus for the production of amylases (Pandey et al., 1999, 2005). In the production of

amyloglucosidase, the species *Aspergillus niger*, *A. oryzae, A. awamori, Fusarum oxysporum*, *Humicola insolens*, *Mucor pusillus*, *Trichoderma viride* . Species Are producing α-amylase. *Aspergillus niger*, *A. fumigatus*, *A. saitri, A. terreus*, *A. foetidus foetidus*, *Rhizopus, R. delemar* (Pandey et al. 2005), with special emphasis on the species of the genera *Aspergillus* sp., *Rhizopus* sp. and *Endomyces* sp (Soccol et al. 2003).

In fact, filamentous fungi and the enzymes produced thereby have been used in food and in the food-processing industries for decades. In fact, their GRAS (Generally Recognized as Safe) status is acknowledged by the U.S. Food and Drug Administration in the case of some species such as *Aspergillus niger* and *Aspergillus oryzae*.

The food industry use amylases for the conversion of starch into dextrins. The latter are employed in clinical formulas as stabilizers and thickeners; in the conversion of starch into maltose, in confectioneries and in the manufacture of soft drinks, beer, jellies and ice cream; in the conversion of starch into glucose with applications in the soft-drinks industry, bakery, brewery and as a subsidy for ethanol production and other bioproducts; in the conversion of glucose into fructose, used in soft drinks, jams and yoghurts (Aquino et al., 2003, Nguyen *et al.*, 2002).

Amylases provide better bread color, volume and texture in the baking industry. The use of these enzymes in bread production retards its aging process and maintains fresh bread for a longer period. Whereas fungal α-amylase provides greater fermentation potential, amyloglucosidase improves flavor and taste and a better bread crust color (Novozymes, 2005). Amylases are the most used enzymes in bread baking (Giménez et al. 2007; Haros; Rosell, Leon; Durán).

Amylases have an important role in carbon cycling contained in starch by hydrolyzing the starch molecule in several products such as dextrins and glucose. Dextrins are mainly applied in clinical formulas and in material for enzymatic saccharification. Whereas maltose is used in confectioneries and in soft drinks, beer, jam and ice cream industries, glucose is employed as a sweetener in fermentations for the production of ethanol and other bioproducts.

The above amylases break the glycosidic bonds in the amylose and amylopectin chains. Thus, amylases have an important role in commercial enzymes. They are mainly applied in food, drugs, textiles and paper industries and in detergent formulas (Peixoto et al. 2003; Najafpour, Gupta et al., 2002; Asghar et al. 2006; Mitidieri et al., 2006).

Results from strains tested for the potential production of amylases, kept at 4°C during 10 days, indicated that the wild and mutant strains still removed the nutrients required from the medium by using the available substrate. This fact showed that cooling maintained intact the amylase's activities or that a stressful condition for the fungus caused its degradation and thus consumed more compounds than normal (Smith, et al., 2010).

The best enzyme activity of microbial enzymes occurs in the same conditions that produce the microorganisms' maximum growth. Most studies on the production of amylases were undertaken from mesophilic fungi between 25 and 37°C. Best yields for α-amylase were achieved between 30 and 37°C for *Aspergillus* sp.; 30°C for *A. niger* in the production of amyloglucosidase 30°C in the production of α-amylase by *A. oryzae* (Tunga, R.; Tunga B.S, 2003), 55°C by thermophile fungus *Thermomonospora*, and 50°C by *T. lanuginosus* in the production of α-amylase (Gupta et al., 2003). However, no reports exist whether increase in enzyme activity after growth of fungus in ideal conditions and kept refrigerated at 4°C for 10 days has ever been tested.

2.1.6 Proteases

Proteases are enzymes produced by several microorganisms, namely, *Aspergillus niger, A. oryzae, Bacillus amyloliquefaciens*, *B. stearotermophilus*, *Mucor miehei, M. pusillus*. Proteases have important roles in baking, brewing and in the production of various oriental foods such as soy sauce, miso, meat tenderization and cheese manufacture.

Man's first contact with proteases activities occurred when he started producing milk curd. Desert nomads from the East used to carry milk in bags made of the goat's stomach. After long journeys, they realized that the milk became denser and sour, without understanding the process's cause. Curds became thus a food source and a delicacy. Renin, an animal-produced enzyme, is the protease which caused the hydrolysis of milk protein.

Proteases, enzymes that catalyze the cleavage of peptide bonds in proteins, are Class 3 enzymes, hydrolases, and sub-class 3.4, peptide-hydrolases. Proteases may be classified as exopeptidases and endopeptidases, according to the peptide bond to be chain-cleaved.

Recently proteases represent 60% of industrial enzymes on the market, whereas microbial proteases, particularly fungal infections, are advantageous because they are easy to obtain and to recover (Smith et al, 2009).

An enzyme extract (Neves-Souza, 2005), which coagulates milk and which is derived from the fungus *Aspergillus niger* var. *awamori*, is already produced industrially.

Although the bovine-derived protease called renin has been widely used in the manufacture of different types of cheese, the microbial-originated proteases are better for coagulant (CA) and proteolytic (PA) activities (PA). The relationship AC / AP has been a parameter to select potentially renin-producing microbial samples. The higher the ratio AC / AP, the most promising is the strain. It features high coagulation activity, with fewer risks in providing undesirable characteristics from enhanced proteolysis (Melo et al, 2002).

The microbial proteases have also been important in brewery. Beer contains poorly soluble protein complexes at lower temperature, causing turbidity when cold. The use of proteolytic enzymes to hydrolyze proteins involved in turbidity is an alternative for solving this problem.

Most commercial serine proteinases (Rawling et al, 1994), mainly neutral and alkaline, are produced by organisms belonging to the genus *Bacillus*. Whereas subtilisin enzymes are representatives of this group, similar enzymes are also produced by other bacteria such as *Thermus caldophilus*, *Desulfurococcus mucosus* and *Streptomyces* and by the genera *Aeromonas* and *Escherichia coli*.

In their studies and observations on the activities of proteases from *Bacillus*, Singh and Patel (2005), Silva, and Martin Delaney (2007); Sheri and Al-Mostafa (2004) and others evaluated their properties for a better performance in pH and temperature ranges.

2.1.7 Glucose oxidase

Glucose oxidase [E.C. 1.1.3.4] (GOx) is an enzyme that catalyzes the oxidation of beta-D-glucose with the formation of D-gluconolactone. The enzyme contains the prosthetic group flavin adenine dinucleotide (FAD) which enables the protein to catalyze oxidation-reduction reactions.

Guimarães et al. (2006) performed a screening of filamentous fungi which could potentially produce glucose-oxidase. Their results showed high levels of GOx in *Aspergillus versicolor* and *Rhizopus stolonifer*. The literature already suggests that the genus *Aspergillus* is a major GOx producer.

The enzyme is used in the food industry for the removing of harmful oxygen. Packaging materials and storage conditions are vital for the quality of products containing probiotic microorganisms since the microbial group's metabolism is essentially anaerobic or microaerophilic (MattilaSandholm et al., 2002). Oxygen level during storage should be consequently minimal to avoid toxicity, the organism's death and the consequent loss of the product's functionality.
Glucose oxidase may be a biotechnological asset to increase stability of probiotic bacteria in yoghurt without chemical additives. It may thus be a biotechnology alternative.

2.1.8 Glucose isomerase

Glucose isomerase (GI) (D-xylose ketol isomerase; EC 5.3.1.5) catalyzes the reversible isomerase from D-glucose and D-xylose into D-fructose and D-xylulose, respectively. The enzyme is highly important in the food industry due to its application in the production of fructose-rich corn syrup.
Interconversion of xylose into xylulose by GI is a nutritional requirement of saprophytic bacteria and has a potential application in the bioconversion of hemicellulose into ethanol. The enzyme is widely distributed among prokaryotes and several studies have been undertaken to enhance its industrial application (Bhosale et al, 1996).
The isolation of GI in *Arthrobacter* strains was performed by Smith et al. (1991), whereas Walfridsson et al. (1996) cloned gene xylA of *Thermus themophilus* and introduced it into *Saccharomyces cerevisiae* to be expressed under the control of the yeast PGK1 promoter. The search for GI thermostable enzymes has been the target of protein engineering (Hartley et al., 2000).
In fact, biotechnology has an important role in obtaining mutants with promising prospects for the commercialization of glucose isomerase enzyme.
The development of microbial strains which use xylan with prime matters for the growth or selection of GI-constituted mutants should lead towards the discontinuation of the use of xylose as an enzyme production inducer.

2.1.9 Invertase

Invertase is an S-bD-fructofuranosidase obtained from *Saccharomyces cerevisiae* and other microorganisms. The enzyme catalyzes the hydrolysis from sucrose to fructose and glucose. The manufacture of inverted sugar is one of invertase's several applications. Owing to its sweetening effects which are higher than sucrose's, it has high industrial importance and there are good prospects for its use in biotechnology.
Invertase is more active at temperatures and pH ranging respectively between 40º and 60º C and between 3.0 and 5.0. When invertase-S is applied at 0.6% rate in a solution of sucrose 40% w / w at 40°C, it inverts 80% of sucrose after 4h. 20min.
When Cardoso et al. (1998) added invertases to banana juice to assess its sweetness potential, they reported an increase in juice viscosity besides an increase in sweetness.
Alternaria sp isolates from soybean seed were inoculated in a semi-solid culture and the microorganism accumulated large amounts of extracellular invertase, which was produced constitutively without the need for an inductor.
Microorganisms, such as filamentous fungi, are good producers of invertase with potential application in various industrial sectors.
Gould et al. (2003) cultivated the filamentous fungus *Rhizopus* sp in wheat bran medium, and obtained invertase identified as polyacrylamide gel. Another potentially producing

fungus invertase is *Aspergillus casiellus*. It was inoculated in soybean meal medium and after 72 hours its crude extract was isolated (Novak et al., 2010). Since most invertases used in industry are produced by yeasts, underscoring the search for fungi that produce it in great amounts is a must.

3. Final considerations

Perspectives for biotechnological production of enzymes by microorganisms.

Biotechnology is an important tool for a more refined search for microorganisms with commercial assets. Microorganisms have existed on the planet Earth during millions of years and are a source of biotechnological possibilities due to their genetic plasticity and adaptation.

The isolation of new species from several and different habitats, such as saltwater and freshwater, soils, hot springs, contaminated soils, caves and hostile environments is required. Microorganisms adapted to these conditions may have great biotechnological potential.

Methods such as the selection of mutants are simple ways to obtain strains or strains with enzymatic possibilities and these methods are widely used by researchers in academic pure science laboratories.

Geneticists also employ the recombination and selection of mutants, which feature promising characteristics, in new strains. This method consists of transferring genetic material among contrasting genotypes, obtain recombinants and use the selection for the desired need.

The recombinant DNA technology (TDR) is a very useful method under three aspects: it increases the production of a microbial enzyme during the fermentation process; it provides enzymes with new properties suitable for industrial processes, such as thermostability and ability to function outside the normal pH range; it produces enzymes from animal- and vegetable-derived microorganisms.

Extremophile microorganisms are potentially producing enzymes with useful characteristics for high temperature industrial processes.

Microorganisms that grow at low temperatures have important biotechnological assets since their enzymes are more effective at low temperatures and enables contamination risks in continuous fermentation processes. This will shorten fermentation time and enhance energy saving.

The DNA sequencing technologies have advanced greatly in recent years and important progress on genes that synthesize proteins and thus determine their function in organisms has been achieved.

Genomes of several microorganisms have been sequenced, including those which are important for the food industry, such as *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactococcus Latis*, *Lactobacillus acidophilus*, *Lactobacillus* sp, and *Streptococcus thermophilus*. These genomes have revealed several new genes, most of which codify enzymes.

Microorganisms are potential producers of enzymes useful for the food industry. Biotechnological tools are available for the selection and obtaining of strains and for strains which increase enzymes' production on a large scale.

Progress and achievements in this area will bring improvements in the food industry and, consequently, a better health quality for mankind.

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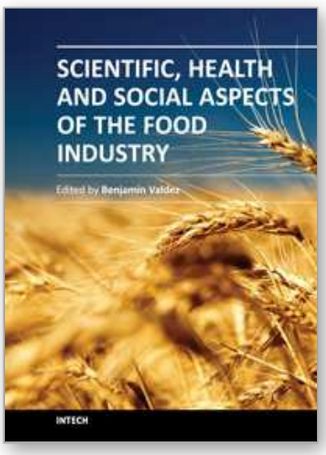
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This book presents the wisdom, knowledge and expertise of the food industry that ensures the supply of food to maintain the health, comfort, and wellbeing of humankind. The global food industry has the largest market: the world population of seven billion people. The book pioneers life-saving innovations and assists in the fight against world hunger and food shortages that threaten human essentials such as water and energy supply. Floods, droughts, fires, storms, climate change, global warming and greenhouse gas emissions can be devastating, altering the environment and, ultimately, the production of foods. Experts from industry and academia, as well as food producers, designers of food processing equipment, and corrosion practitioners have written special chapters for this rich compendium based on their encyclopedic knowledge and practical experience. This is a multi-authored book. The writers, who come from diverse areas of food science and technology, enrich this volume by presenting different approaches and orientations.

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