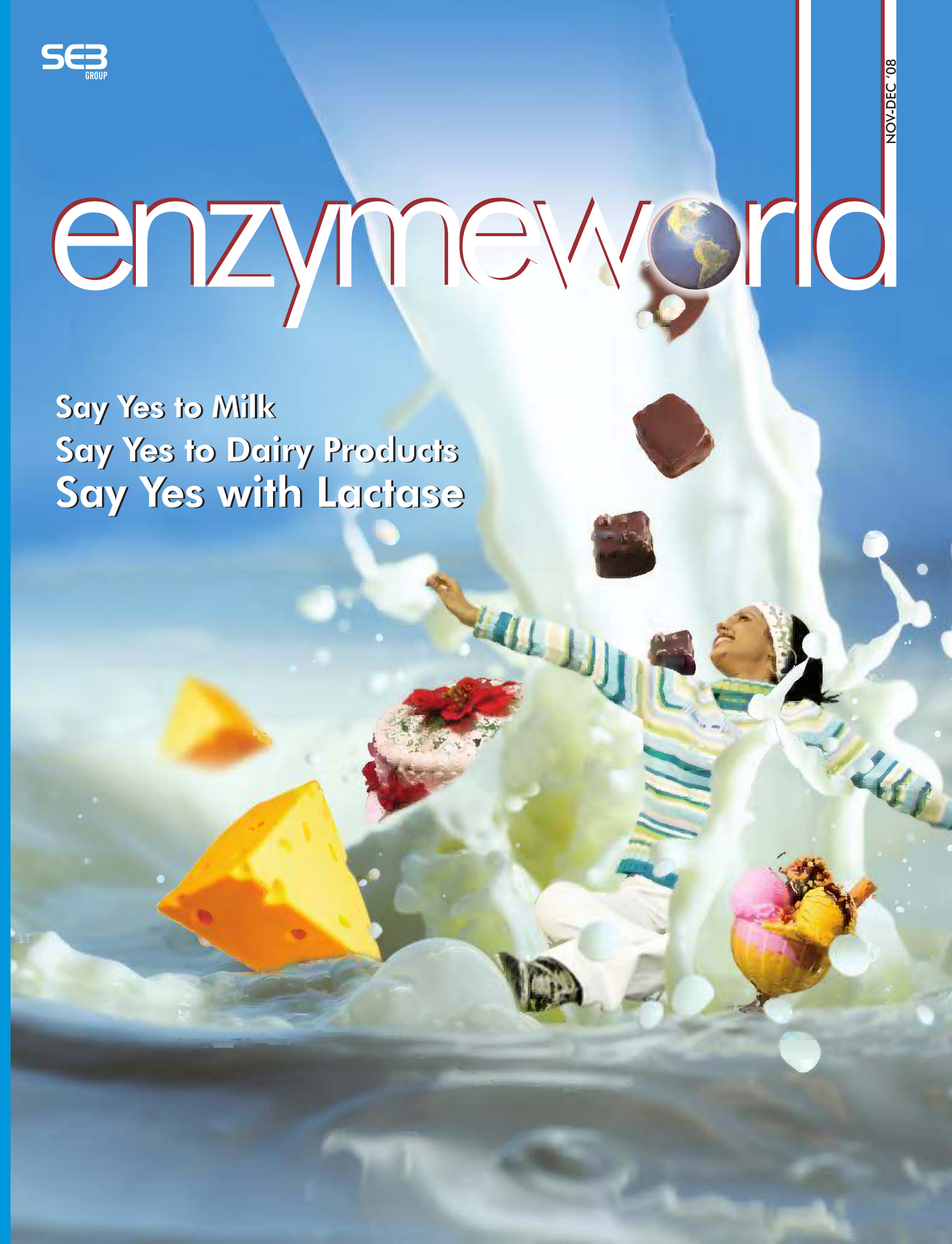


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QUIZ

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**Question 1: Enzymes get deactivated when exposed to**

- a) Optimum pH
- b) Excessive heat
- c) Sodium ions
- d) Calcium ions

**Question 2: The natural gas buster is**

- a) Protease
- b) Catalase
- c) Hemicellulase
- d) Lipase

**Question 3: Lactase acts on.**

- a) Milk Sugar
- b) Carbohydrate
- c) Cellulose
- d) None of the above.

**Question 4: Bile supports**

- a) Amylase
- b) Carbohydrates
- c) Pancreatin
- d) Cellulase

**Question 5: Which enzyme digests hydrogen peroxide into Oxygen bubbles and water, releasing heat in the process?**

- a) Amylase
- b) Catalase
- c) Lipase
- d) Pepsin

**Question 6: Lipases can be classified as**

- a) Transferases
- b) Oxidoreductases
- c) Hydrolases
- d) Isomerases

**Question 7: Probiotics are**

- a) Human friendly microbes
- b) Pathogens
- c) Disease causing
- d) None of Above

**Question 8: Enzyme work by lowering**

- a) Chemical energy
- b) Free energy
- c) Bond energy
- d) Solvent energy

**Question 9: Deseizing of the fabric is done by enzyme**

- a) Amylase
- b) Protease
- c) Phytase
- d) None of Above

**Question 10: The enzymes exhibit activity through**

- a) Energy Site
- b) Active Site
- c) Free site
- d) None of above.

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## NEWS BRIEFS

### FDA Warns Companies to Stop Marketing Topical Drug Products Containing Papain

The U.S. Food and Drug Administration (FDA) today announced that companies marketing unapproved ophthalmic balanced salt solutions (BSS) and unapproved topical drug products containing papain must stop manufacturing and marketing these products or risk enforcement action. FDA is taking these actions because it has received reports of serious adverse events associated with their uses.

Companies marketing any unapproved topical drug products containing papain must stop manufacturing them on or before November 24, 2008. Companies or others engaged in shipping these products must stop shipping these products on or before Jan. 21, 2009. After these dates, all topical products containing papain must have FDA approval to be manufactured or shipped in interstate commerce. Companies that continue to market unapproved topical papain products after these dates may be subject to immediate FDA enforcement action, such as seizure and/or injunction against the company. At this time it is unclear whether other enzymes like bromelain will be similarly affected.

### Country of Origin Labeling (COOL)

#### COOL Implementation: Legislative History and Status of Rulemaking

The U.S. 2002 and 2008 Farm Bills amended the Agricultural Marketing Act of 1946 to require retailers to notify their customers of the country of origin of beef, lamb, pork, chicken, goat, wild and farm-raised fish and shellfish, perishable agricultural commodities, peanuts, pecans, ginseng, and

macadamia nuts. The implementation of mandatory COOL for all covered commodities except wild and farm-raised fish and shellfish was delayed until September 30, 2008. The law defines the terms "retailer" as any person engaged in the business of selling any perishable agricultural commodity at retail. Retailers are required to be licensed when the invoice cost of all purchases of perishable agricultural commodities exceeds \$230,000 during a calendar year. The term perishable agricultural commodity means fresh and frozen fruits and vegetables. Food service establishments are specifically exempted as are covered commodities that are ingredients in a processed food item. In addition, the law specifically outlines the criteria a covered commodity must meet to bear a "United States country of origin" designation.

### Diabetes Increases at an Alarming Rate

The World Health Organization statistics have estimated 246 million people worldwide are affected by diabetes. This does not even consider those that are pre-diabetic. The rate of new cases is expected to exceed 7 million new cases a year, resulting in at least 389 million diabetics by 2025. Currently, it is the seventh leading cause of death and the leading cause of new blindness and kidney failure. Patients and health care providers are ill equipped to handle the complexities of a disease that is rapidly challenging worldwide health care systems, particularly in North America where 2/3 of the population is overweight. This is a true health crisis that goes well beyond successfully regulating blood glucose? The healthcare costs are staggering and continue to grow.



## Editorial

Welcome to the November/December issue of Enzyme World. This issue reaches you at a time of worldwide economic distress. Everywhere there is concern for the future. Yet, people will continue to need food, clothes, fuel and other products and services. Therefore, manufacturers must find ways to gain the greatest efficiency for with the least expense necessary. In other words, the biggest return possible relative to expenditures. It is clear that enzymes can significantly improve efficiency and yield with minimal cost outlays. It is our editorial goal to provide our readers with articles that provide information that will help in these areas. For specific questions, please feel free to contact us at our "Ask the Enzyme Experts" email, ew@enzymeindia.com and one of our experts will contact you directly.

In this issue of Enzyme World, we will cover the state of ethanol production worldwide. In addition, we have a timely article on the use of lactase enzyme for those who have difficulty digesting dairy products. Finally, we have a discussion on the one common denominator of all plants, a cell wall. The cell wall is basic to processing things as diverse as fruit juice, ethanol, paper and textiles. In future issues, we will continue to provide a diverse range of applications where enzymes provide a solution. We hope each issue of Enzyme World will provide useful enzyme solutions for you.

### LACTASE

Difficulty digesting dairy products is a relatively common problem. We all like ice cream, cheese, yogurt and milk shakes, yet for many, the price is indigestion, gas, abdominal cramping and more. The most common cause is lactose intolerance. While lactose intolerance varies wildly from population to population, scientists estimate that nearly 75% of adults either lack the ability to digest lactose or at least have difficulty digesting dairy products in general. The frequency ranges from around 5% in northern Europe to more than 90% in some Asian and African countries. There is a simple answer in the form of lactase enzyme. This article discusses the mechanics of its action.

### THE CELL WALL AND ENZYME EXTRACTION

#### OF FRUITS, VEGETABLES AND GRAINS

Enzymes are extremely important in processing fruit, vegetables and grains. Both the quality and the yield are significantly improved with the proper use of enzymes. In order to understand why enzymes are important, it is necessary to understand the one common denominator of all plants: the cell wall. Unlike animals, plant cells have a non-living cell wall with a living cell membrane in the interior. The cell wall is immensely complex containing an array of carbohydrates including cellulose, hemicellulose, pectin and in the case of cereal grains, beta glucans. Of the remaining molecules, the most important is a non-saccharide molecule called lignin. The very complexity of the cell wall presents real difficulties when it comes to processing fruit and grain in particular.

### FUEL ETHANOL SCENARIO

Fuel Ethanol production in the world is lead by the U.S. and Brazil followed by China and India. New ethanol producers continue to emerge throughout Asia and Latin America, among others. The substrates used vary from corn and other grains, sugar cane, molasses and plant source cellulose. The use of corn as a substrate is somewhat controversial due to its potential impact on food costs. Cost effective production of ethanol from a cellulosic source is still considered the ultimate goal. As yet, cellulosic production is still somewhat problematic. It is estimated the world production of ethanol will reach about 75 to 80 billion liters by 2012.

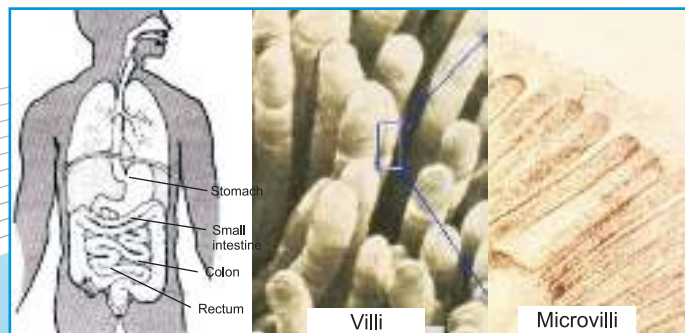
The SEB Companies, Advanced Enzymes and Specialty Enzymes, have as part of our mission statement, a responsibility to provide industry with enzyme solutions that are not only effective, but help, not harm the environment. Our company continues to look for novel product solutions that reduce or eliminate pollution of the environment. In essence, we strive to provide a value added product line that is an eco-friendly solution as well.

Read on,  
Mike Smith

## Lactase Helps You Enjoy the Benefits of Milk

Lactose intolerance is the inability to metabolize milk sugar, or lactose. Lactose is a disaccharide found in dairy products and is composed of glucose and galactose. While lactose intolerance varies wildly from population to population, scientists estimate that nearly 75% of adults either lack the ability to digest lactose or at least have difficulty digesting dairy products in general. The frequency ranges from around 5% in northern Europe to more than 90% in some Asian and African countries.

The issue of whether we can digest lactose depends upon the intestinal enzyme Lactase. Lactase (or beta-galactosidase) is the enzyme that hydrolyzes lactose into its component monosaccharides, galactose and glucose. Lactase is naturally produced in the small intestine. In the inner lumen of the small intestine, projections called villi are covered with cells known as enterocytes. These enterocytes are covered with a membrane that has what is called a brush border. The brush border is actually millions of microvilli and is the site of lactase production.

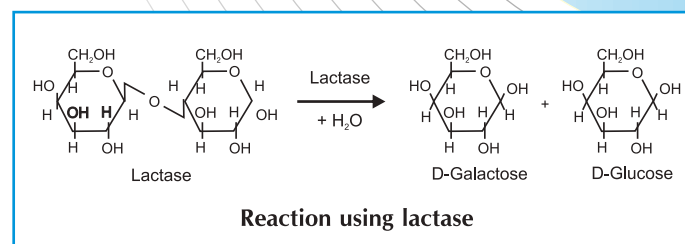


Villi are projections into the lumen of the small intestine that are covered with enterocytes. An electron micrograph of the microvilli of an enterocyte with brush border is shown

Villi are projections into the lumen of the small intestine that are covered with enterocytes. An electron micrograph of the microvilli of an enterocyte with brush border is shown

Lactase promotes the hydrolysis of the Beta-D galactoside linkage of lactose, converting it into D-glucose and D-galactose. This reaction provides two positive results for dairy products, which we will cover later in the article. Lactase is very active in the duodenum. Its ability to handle lactose loads is very closely related to the amount of lactose emptying from the stomach into the duodenum.

The optimum temperature for lactase is about 48 °C (118.4 °F) for its activity and has an optimum pH of 6.5.



Lactase produced commercially is commonly extracted from yeast fungi such as *Kluyveromyces fragilis*. Other known sources include other yeasts, certain bacteria and fungi. The lactase enzyme



### Cellulosic Ethanol:

This is a complex area by virtue of the range of raw materials that are likely to be used, the differences in their chemical compositions and the degree of complexity of their structural construction. Therefore, the research that is going on encompasses several areas which include

- a) Engineering plants / trees that will have less complex cellular structure and permit easier conversion to fermentable sugars.
- b) Physical, chemical and thermal pre treatment techniques for separation of lignin from biomass and easier conversion of cellulose and hemicellulose to fermentable sugars.
- c) Developing more efficient enzyme systems that are capable of releasing fermentable sugars more completely, easily and much faster.
- d) Reducing the cost of production of enzyme systems.
- e) Converting lignin to ethanol increasing profitability of cellulosic ethanol.
- f) Engineering yeasts and/or bacteria that ferment both pentoses and hexoses into ethanol.
- g) Engineering yeasts and other microorganisms to co-produce ethanol and other high value



molecules at the same time.

- h) Depolymerization of lignin to high value chemicals.

An enormous amount of research is going on in the area of cellulosic ethanol with several new ideas cropping up almost everyday. A recent discovery by Dr. Susan Leschine, professor of microbiology at the University of Massachusetts Amherst holds some promise to improve cellulosic ethanol production. The microbe was discovered in soil

near a Massachusetts reservoir and named Q-Microbe. It is a naturally occurring soil bacterium (*Clostridium phytofermentans*) that appears to be able to convert both pentoses and hexoses directly into ethanol.

No doubt, other organisms will arise, whether by natural

means or through bioengineering. Cellulosic ethanol could become a common entity within the next two years time.

Advanced Enzyme Technologies Ltd together with its Group Company Specialty Enzymes and Biochemicals, is involved in finding ways to add value to bioethanol production processes with its unique enzymatic solutions.

unavailability of sufficient quantity of ethanol. In order to drive the production of ethanol to required levels, India needs to implement the following.

- a) Market driven flexible pricing of ethanol to make it profitable to produce fuel ethanol
- b) Free movement of molasses across states without interstate levies or taxes
- c) Diversion of excess sugar cane juice to ethanol fermentation and
- d) Development of sweet sorghum with high yielding varieties and improved agricultural practices both for grain, cane as well as cellulosic mass
- e) Opening up of domestic market for imported ethanol and
- f) Development of second generation bio-fuels from cellulosic biomass.

However, in order to be able to reach the level of Brazil in terms of consumption of ethanol as percentage of fuel consumed by light vehicles, aggressive development of cellulosic ethanol is a necessity.

**TECHNOLOGICAL TRENDS AND DEVELOPMENTS:**

**Grain Ethanol:**

Fuel ethanol from grain is predominantly produced by dry grind process and a small part of it is produced by wet milling process. The primary reason for this is that dry grind processes need much less capital investment as compared to wet milling processes. Since fuel ethanol production has taken off with such a sudden boom, industry prefers the

route of lower capital investment.

However, the value realization per ton of grain is much higher in wet milling process as this process recovers many fractions of grain as high value co-products. On the other hand, in dry grind process, the whole grain is milled, slurried and starch portion is converted in to sugars and then to ethanol and the remaining portion is recovered as distillers grain and sold to animal feed. Therefore, the profitability of dry grind ethanol is less. in order to improve the profitability of dry grind process, research is taking place in the following areas:

- a) Recovering the various fractions of grain in pure form during dry grind process as high value co-products and taking only the starch portion for saccharification and fermentation. This will automatically allow more sugars to be fed to fermenters and fermentation cost can come down.
- b) Improving yeast strains that are capable of producing very high concentrations of ethanol up to 23 to 24%.
- c) Developing strategies that can increase the value of by-products of the current dry grind processes.
- d) Reducing energy consumption and improving separation efficiency at various stages in current dry grind processes and
- e) Superior technologies that can recover ethanol at much lower cost.

An important and notable area of research being done also involves production of grains that are capable of self converting their own starch.

produced by these organisms generally has pH optimumsonthealkalinesideorintheweaklyacidpH rangeofabout5-7.

Lactase from the yeast source is stable from pH 6.0 to 8.0 and can be used to hydrolyze lactose in neutral pH products.

Fungal lactase is effective over a lower range, pH 3.5 to 6.5, making it suitable for milk products and whey in the acidic range. The correct lactase concentration is dependent on the initial concentration of lactose, the degree of hydrolysis required, pH, temperature and the time of hydrolysis.

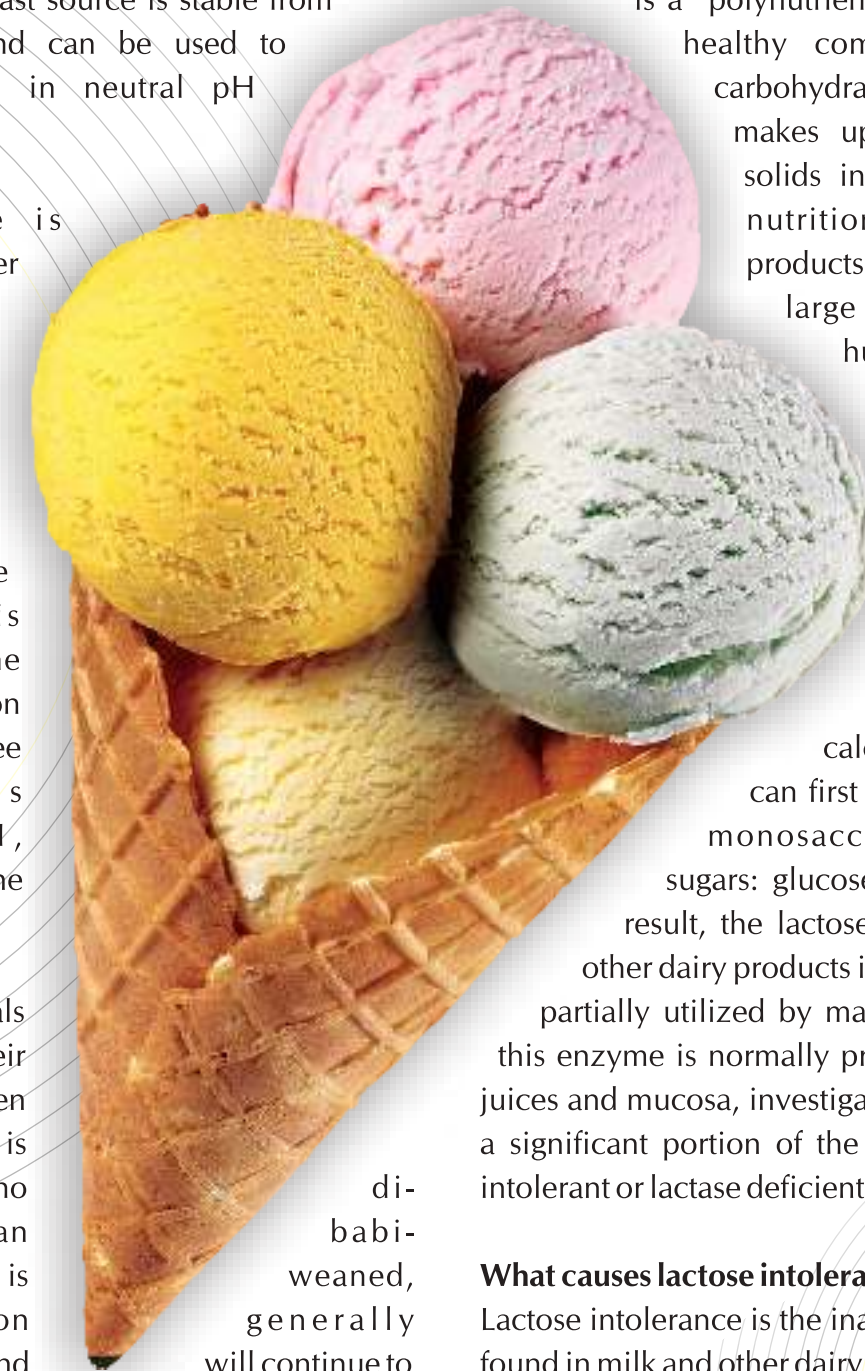
All infant mammals are fed milk by their mothers. This is when lactase production is at its peak. It is no fferent for human es. After a child is lactase production begins to decline and

decrease into adulthood.

Lactose is abundant in milk and dairy products in general such as yogurt, cheese and whey. Milk is a “polynutrient” in that it has many healthy components. Its major carbohydrate is lactose, which makes up from 2-8% of the solids in milk. However, the nutritional value of milk products is limited due to the large proportion of the human population that either lacks or does not produce a de quate quantities of the intestinal enzyme lactase. Humans cannot absorb lactose for use as a calorie source unless they can first break it down into its monosaccharide component sugars: glucose and galactose. As a result, the lactose content of milk and other dairy products is not utilized or is only partially utilized by many persons. Although this enzyme is normally present in the intestinal juices and mucosa, investigations have shown that a significant portion of the population is lactose intolerant or lactase deficient.

**What causes lactose intolerance?**

Lactose intolerance is the inability to digest lactose found in milk and other dairy products. It occurs



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generally  
will continue to



An estimate puts Brazil's domestic ethanol demand in the year 2014 at 32 billion liters and exports at around 7 billion liters. It is estimated that by 2014, ethanol will make up 53 percent of fuels used by light vehicles, due to the increase in the flex-fuel fleets.

The growing demand for ethanol among top importers like the United States and the European Union continues to increase. That coupled with increased production cost of grain ethanol and reduced production cost of cane ethanol in Brazil is expected to help boost Brazilian exports in 2008 and beyond.

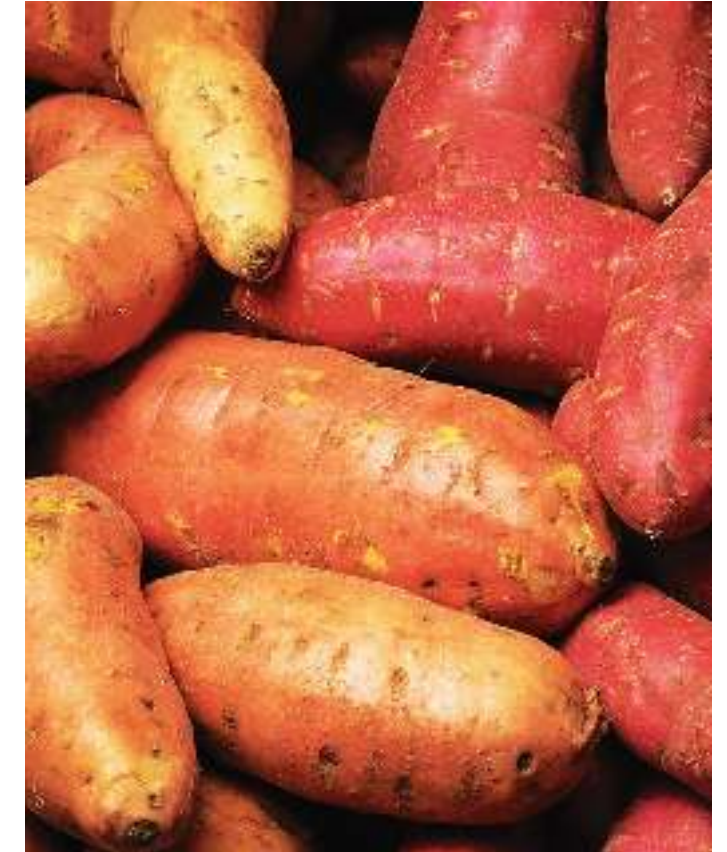
Brazil has successfully proved that bio-fuels do not push up the food prices.

**CHINA:**

The fuel ethanol production in China has increased from 0.378 billion liters in 2004 to 1.89 billion liters in 2007.

The Chinese government views bio-ethanol as a key component of an overall strategy for a stable and secure economy. Increased biofuel production is also seen as a means of mitigating poverty conditions in rural areas, where per capita income is about one-third of that in urban centers. For these reasons the Chinese government continues to take an active role in regulating both supply and demand in the biofuels market.

In response to high food prices, the government has suspended new corn-based ethanol projects, including any plans to expand existing plants. The aim is to shift production of fuel ethanol from grains to other feedstocks such as sweet potato, cassava,



and sweet sorghum. China is also focusing on development of cellulosic ethanol.

**INDIA:**

Fuel ethanol in India is produced from molasses and the production in 2007 was about 670 million litres. This is sufficient enough to meet the requirements of E5 (5% ethanol in gasoline) which is the first stage of fuel ethanol program of Government of India.

The Government of India has proposed to implement E10 from 2008 and E20 from 2017. This puts the ethanol requirement at 1.4 billion liters in 2008 - '09 and at 5 billion liters in 2017. The implementation of E10 is delayed due to

when the small intestine does not produce enough lactase.

Primary lactase deficiency is a condition that develops over time. After about age 2 the body begins to produce less lactase, though most people will not notice symptoms until much later. Some are born with a genetic predisposition to develop primary lactase deficiency.

Secondary lactase deficiency occurs when injury to the small intestine or certain digestive diseases reduce the amount of lactase a person produces. These diseases include celiac disease, inflammatory bowel disease, Crohn's disease, gastroenteritis, or as a result of gastroduodenal surgery.

Temporary lactase deficiency can result from viral and bacterial enteritis, especially in children, when

the mucosal cells of the intestine are injured. Researchers have identified a genetic link for lactose intolerance.

**Who is at risk?**

Between 30 and 50 million Americans are lactose intolerant and certain ethnic and racial populations are more affected than others. Up to 80 percent of Africans, 80 to 100 percent of American Indians, and 90 to 100 percent of Asians are lactose intolerant. The condition is least common among people of northern European descent. Premature infants are more likely to be lactose intolerant since lactase levels do not increase until the third trimester of pregnancy.

**Symptoms:**

People with lactose intolerance may experience abdominal



In addition, grain ethanol generates employment in rural areas in addition to several other benefits. The Energy Independence and Security Act of 2007 of US requires the industry to produce 36 billion gallons of ethanol by 2022. A large portion of this is expected to come out of cellulosic ethanol. Quite a few demonstration plants are under construction to validate the technical and financial models for cellulosic ethanol in USA. An enormous amount of research, focused in several angles is going on in USA to convert the cellulosic biomass completely in to useful products in simpler and cheaper ways than is known today.

The Energy Independence and Security Act of 2007 also aims at reducing U.S. demand for oil by setting a national fuel economy standard of 35 miles per gallon by 2020.

In the meantime for those driving flex-fuel vehicles in US, it is still tough to find E85 (automobile fuel with 85% ethanol) in most of the country. There are an estimated 1,200 E85 filling stations in the U.S. as of now with the heaviest concentrations in the Midwest region. As of now, most of the ethanol being produced is blended into gasoline at low concentrations to produce E10 which can be used in all cars and trucks.

**BRAZIL:**

Brazil produces fuel ethanol from sugar cane. Strong and growing sales of flex-fuel cars have created a record demand for ethanol in Brazil in 2007 and the trend is expected to continue again in 2008, as new car sales boom. After stagnating for 20 years, Brazil's ethanol consumption rose by 3.7



billion liters to a record 16.7 billion liters in 2007. In 2008, it is estimated to grow by another 2.9 billion liters, following an increase in auto sales.

The production of ethanol during the next crop in 2008 is predicted to reach 24 billion to 25-billion liters, while domestic consumption is put on average at around 20 billion liters. This gives the country a higher ethanol surplus that can be exported. The demand from the domestic market is expected to dominate over the next 5 to 7-years in Brazil. The competitive prices of ethanol in 2007 in filling stations have contributed to a rise in car sales, which in turn has fuelled the domestic demand for ethanol.

bloating, excessive intestinal gas, flatulence, nausea, diarrhea, and abdominal cramping. These symptoms may occur anywhere from 30 minutes to two hours after ingesting dairy products. The severity of symptoms usually depends on the amount of lactose ingested and how much of the enzyme, lactase remains in the intestinal tract.

**How is lactose intolerance treated?**  
Lactose intolerance is easy to treat. No treatment can improve the body's ability to produce lactase, but symptoms can be controlled through diet and supplemented lactase. For those who react to very small amounts of lactose and/or have trouble limiting their intake of dairy foods, lactase enzyme can significantly help digestion and reduce symptoms associated with lactose consumption. The lactase supplements are taken with the first bite of dairy food. Lactase enzyme is also available as a liquid. Adding a few drops of the enzyme makes lactose more digestible for people with lactose intolerance.

**Other uses:**  
Lactase is commonly used in the production of ice cream and sweetened, flavored or condensed

milks. When lactase is added to milk or liquid whey (2000 U/kg) and left for about a day at 5°C, about 50% of the lactose is hydrolyzed, giving a sweeter product, which will not crystallize if condensed or frozen. This method enables otherwise-wasted whey to replace some or all of the skim milk powder used in traditional ice cream recipes. It also improves the 'scoopability' and creaminess of the product. Smaller amounts of lactase may be added to long-life sterilized milk to produce a relatively inexpensive lactose-reduced product (e.g. 20 U/kg, 20°C, 1 month of storage).

When lactase is used in the manufacture of ice cream, not only does it effectively reduce crystallization when frozen, but also the glucose and galactose produced are sweeter than lactose providing a sweeter, more pleasant taste. Lactose will crystallize at the low temperatures. Thus, lactase treated ice cream's constituent products stay liquid and contribute to a smoother texture.

The reality is that in most cultures today, dairy products are a major source of nutrition. Whether it is milk, cheese, ice cream, yogurt or whey, lactase offers greater nutritional value to the three-quarters of the world population that currently cannot digest or poorly digests dairy products.



## The Cell Wall and Enzyme Extraction of Fruits, Vegetables and Grains

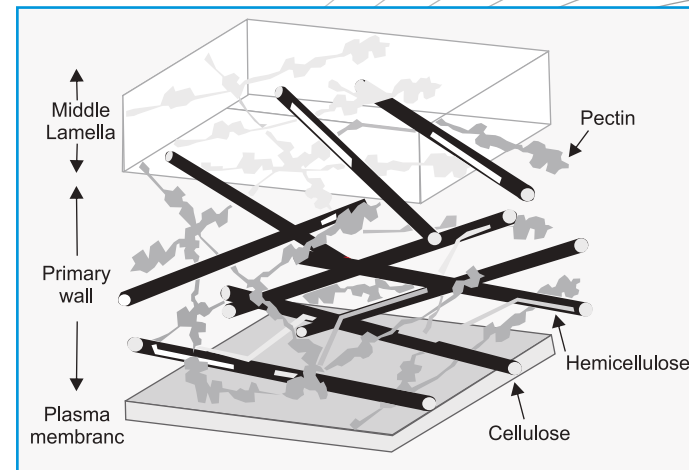
## Celluloic Ethanol Makes Way into Our Lives

One of the more important uses for enzymes is in processing fruit, vegetables and grains. This ranges from the production of fruit juice to distilled spirits. Both the quality and the yield are significantly improved with the proper use of enzymes. Fruit juice is clearer and sweeter. Wine, beer and alcohol production are all significantly enhanced with enzymes. In order to understand why enzymes are important, it is necessary to understand the one common denominator of all plants: the cell wall.

Unlike animals, plant cells have a non-living cell wall with a living cell membrane in the interior. The cell wall has several functions: structural support, shape, control of growth and ultimately the shape of the plant. The cell wall is made up predominantly of carbohydrates: cellulose, hemicellulose, pectin and in the case of cereal grains, beta glucans. Of the remaining molecules, the most important is a non-saccharide molecule called lignin. The cell wall serves an essential service for the plant, but presents real difficulties when it comes to processing fruit and grain in particular.

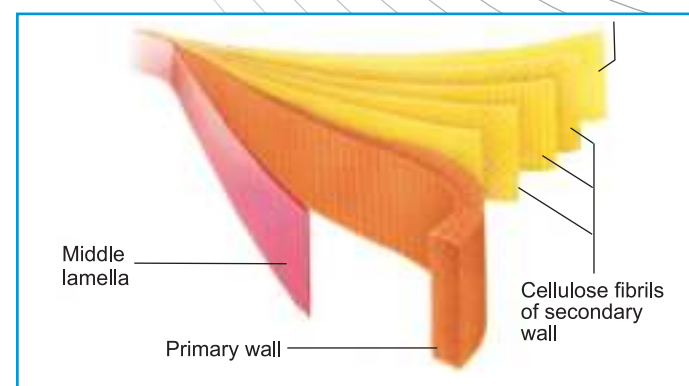
### THE CELL WALL HAS 3 DIFFERENT LAYERS

1. Middle lamella: This is the first layer formed during cell division. It makes up the outer wall of the cell and is the shared intercellular material cementing the primary cell walls of adjacent cells. It is composed of pectic compounds and protein.
2. Primary wall is formed after the middle lamella and consists of a rigid skeleton of cellulose



microfibrils embedded in a gel-like matrix of pectic compounds, hemicellulose, and glycoproteins.

3. Secondary wall: formed after cell enlargement is completed. The secondary wall is extremely rigid and provides compression strength. It is made of cellulose, hemicellulose and lignin. The secondary wall is often layered. As plant cells grow, they deposit new layers of cellulose adjacent to the plasma membrane. Thus the oldest layers are in the primary wall (the outer wall) and in the middle lamella.



### FUEL ETHANOL SCENARIO

Fuel Ethanol production in the world is lead by U.S. and Brazil followed by China and India. New producers continue emerging throughout Asia and Latin America. It is estimated the world production of ethanol will reach about 75 to 80 billion liters by 2012.

#### The main drivers of ethanol production are,

- Limited availability and continued depletion of fossil fuels.
- National energy security considerations.

- High oil prices.
- Ethanol tax incentives.
- Improved technology - lower costs of ethanol production.
- Climate change concerns.

#### USA:

USA produces fuel ethanol primarily from corn and milo.

The world's largest producer of ethanol, the United States of America produced 6.5 billion gallons in 2007 which is 32% more than its production in 2006. The industry grew from 110 bio-refineries operating in 19 states to 139 bio-refineries in 21 states.

In 2008 an estimated 4 billion gallons of ethanol production capacity will be added from 68 bio-refineries that are either under construction or expanding.

Several new projects have been initiated and once these new projects are completed, the U.S. ethanol industry's capacity will be more than 13 billion gallons of ethanol, which is nearly 10% of the nation's gasoline demand.

Fuel ethanol in US is produced primarily from corn and to a lesser extent, milo (sorghum). Though, there has been a lot of discussion about fuel ethanol production being responsible for increased food prices in US and arguments against grain ethanol, a closer examination does not support this view point. Moreover, about one third of grain used for fuel ethanol production is returned back to animal feed in the form of DDG's (dried distillers grains), which otherwise would have to be filled in by grain.



**Low pectin fruits:**

Cherries, nectarines, pear, pineapple and rhubarb

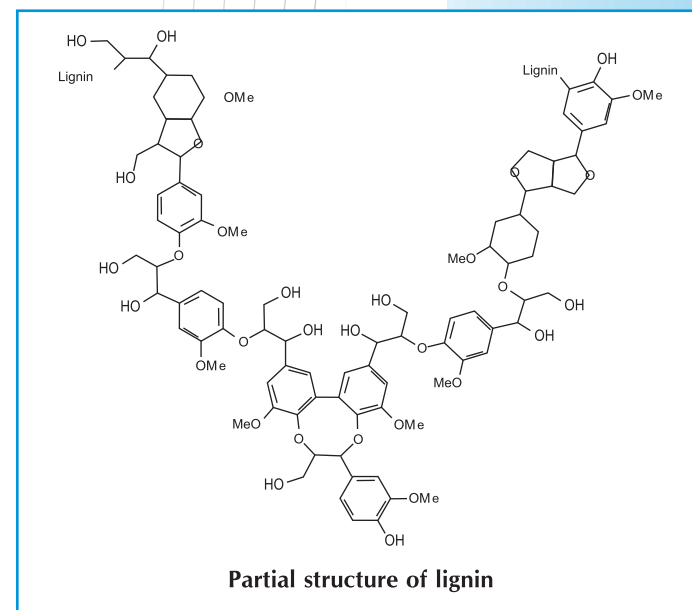
The pectin content in all fruit is also generally higher when fruit is just barely ripe and diminishes as it matures from fully ripe to overripe. The process of ripening involves the breakdown of pectins, which softens the fruit as it ripens. Apples and crabapples (especially unripe ones) are good sources of pectin and are often used in making commercial pectin.

**Structural proteins:** In addition to carbohydrates and lignin, cell walls contain a variety of proteins. Structural proteins are found in all layers of the plant cell wall but they are more abundant in the primary wall layer.

When processing requires fermentation, e.g. beer, wine, distilled spirits and bioethanol, the hydrolysis of protein becomes a very big deal. It is nutritionally important to provide free amino-nitrogen to yeast during fermentation and the free amino-nitrogen comes in the form of the free amino acids released during hydrolysis of protein. Our own SEB-Neutral PL is an ideal protease enzyme for this process.



Lignin is not a carbohydrate; rather it is an aromatic complex of monolignols. It is, nevertheless, a compound that is an integral part of the cell walls of plants. It is one of most abundant organic compounds on earth after cellulose and chitin. Typically, vegetables and fruit possess a lignin content ranging from 1.0 to 6.0%. For example, spinach cell walls contain 4.4% lignin. Lignin fills the spaces in the cell wall between cellulose, hemicellulose and pectin components. Lignin is particularly difficult to digest or even biodegrade. In feed, it reduces the bioavailability of the other cell wall constituents. In paper pulp processing, lignin is typically removed chemically, rather than enzymatically.



As you see, the cell wall is a complex structure that requires very specific enzyme solutions when processing fruits, vegetable and cereal grains. Fortunately, Specialty Enzymes and Advanced Enzymes have the solution.

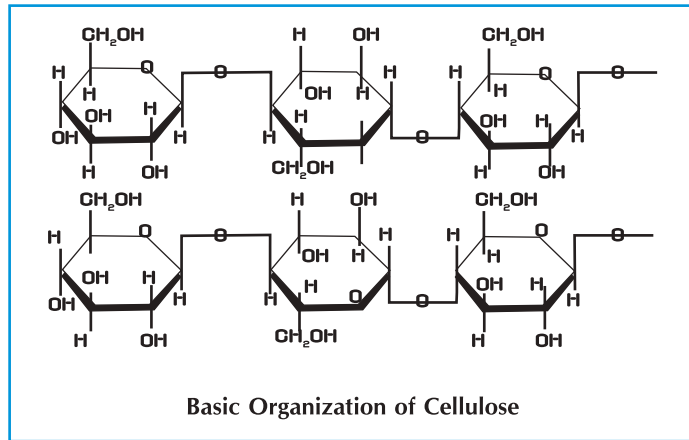


Primary cell walls give shape and structure to plant cells. These walls are sufficiently strong to prevent the cell from rupturing yet must be flexible enough to accommodate growth. Primary walls are believed to consist of several interconnected matrices composed of polysaccharides and glycoproteins. Such matrices include cellulose and associated hemicelluloses (e.g. xyloglucan and galactoglucomannan) and the pectic matrix composed of homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II

**Cellulose:** Humans lack both the enzymes and the microflora necessary to metabolize cellulose. Herbivores like cattle and certain insects like termites have specialized microbes to do the job for them. Dietary supplements may contain enzymes like cellulase, pectinase and hemicellulase to help gain greater nutritional value from plant based

foods. A greater use, however, is in juice, wine, beer and distilled spirits processing. A blend of these enzymes, like those in CelluSEB TL, help increase yields significantly. CelluSEB TL contains a complete array of cell wall degrading enzymes, especially cellulase that helps release vital nutrients present inside the plant cell.

Cellulose is a polymer of glucose - typically consisting of 1,000 to 10,000 beta-D-glucose residues - major component of primary and secondary cell wall layers. Cellulose polymers associate through Hydrogen bonds. The H-bonding of many cellulose molecules to each other results in the formation of microfibrils, which interact with other microfibrils to form fibers. Certain cells, like those in cotton ovules, can grow cellulose fibers of enormous lengths.



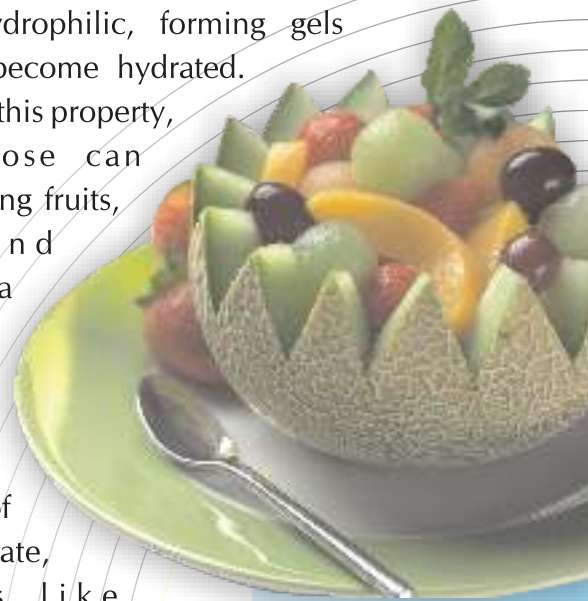
**Hemicellulose:** This is another complex carbohydrate that is an important part of the cell wall. Hemicellulose, along with pectin and cellulose, is present in the cell walls of nearly all plants. Unlike cellulose with its long chain of glucose molecules, Hemicellulose includes a variety of sugars like xylan, arabinoxylan, glucomannan, and xyloglucan, among others. Further, hemicellulose has a lower molecular weight, a more branched and irregular shape.



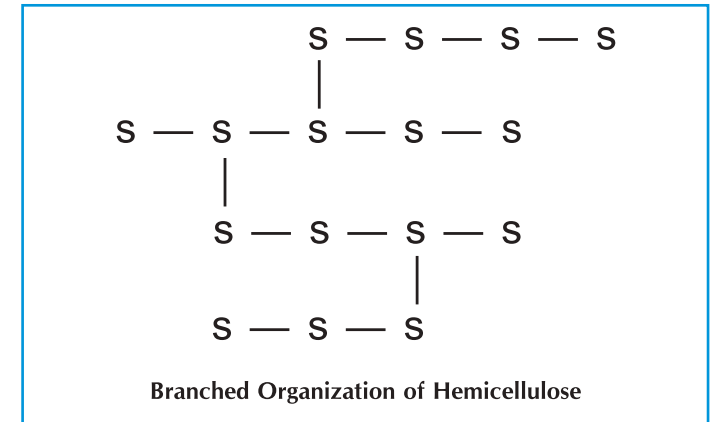
Like the pectin compounds, hemicellulose molecules

are very hydrophilic, forming gels when they become hydrated.

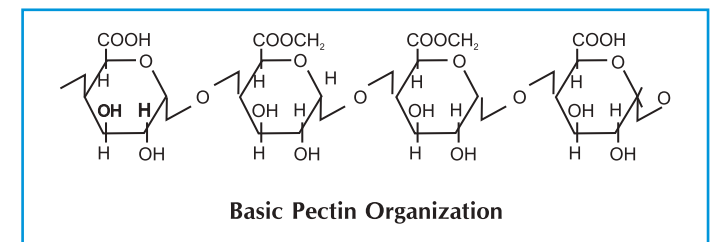
As a result of this property, hemicellulose can make processing fruits, grains and vegetables a thick, viscous mess. Depending on the type of plant substrate, products like CelluSEB TL, SEBflo TL or LiquiSEB RL can improve yield and filtration time.



**Beta Glucan:** The hemicelluloses of plants in the cereal family (Poaceae, formerly Gramineae) are distinct from those of dicots and even other monocots. Many of the most important food crops are cereals, including rice, maize, wheat, oats, barley, and sorghum. Compared to fruits and vegetables, the cereal grain cell walls contain smaller amounts of pectin, large amounts of glucuronoarabinoxylan (GAX) and large amounts of a particular hemicellulose called mixed-linked glucan (MLG) or beta-glucan. Beta glucan is completely absent from other plant species. Beta Glucan makes processing cereal grains a challenge because they make the mash viscous and sticky. A beta glucanase enzyme product like SEBflo TL is the enzyme of choice when processing cereal grain products. SEBflo TL will significantly improve the quality and processing time for grain products such as beer, ale, distilled spirits and bioethanol.



**Pectin:** This is generally a straight chain polymer of D-Galacturonic Acid with alpha-1,4-glycosidic linkage interspersed with another sugar, L-Rhamnose. Pectin is part of the ground substance found in cell walls of plants along with hemicellulose. When processing fruit in particular, pectins tend to make the mash viscous and sticky. Pectinase enzymes like LiquiSEB RL are very effective at reducing viscosity and increasing filtration rates.



**Pectin concentrations vary in fruit.**

**High pectin content fruits:**

Citrus (oranges, tangerines, grapefruit, lemons, limes, etc.), tart cooking apples, Granny Smith apples, cranberries, currants, gooseberries, sour plums, quince and blackberries.

**Medium pectin content fruits:**

Apricots, peaches, rhubarb, strawberries