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# Removal of Excess Cellulose and Associated Polysaccharides in Fruit and Vegetable By-Products – Implication for Use in Feed for Monogastric Farm Animals

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Additional information is available at the end of the chapter

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## 1. Introduction

As an abundant biomaterial, cellulose has many uses. Presently, many investigations are underway to explore its use as fuel. However, cellulose, properly treated, could be used as a source of nutrients for animals. Thus, in this chapter, a case for use of cellulose, accompanying polysaccharides and nutrients in fruit and vegetable by-products (waste) as feed ingredients in animal feed is developed. The production of fruits and vegetables throughout the world and the concomitant loss of by-products are discussed. Potentially, nutrients from by-products could be included in feed for monogastric animals, a source of protein for expanding populations, especially in food insecure parts of the world. The reasons for low use of by-products are delineated with particular attention focused on the indigestibility of lignocellulosic material. Selected treatments to reduce these compounds are examined.

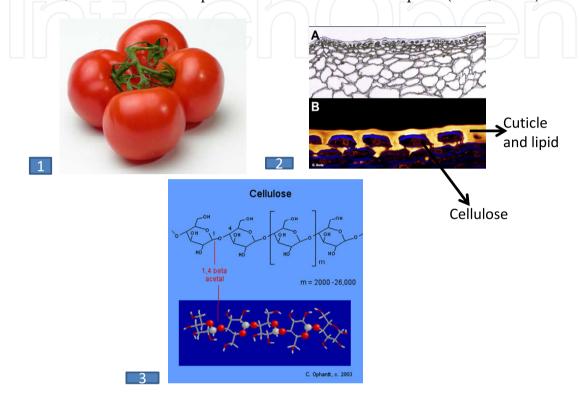
# 2. Worldwide production of cellulose

As shown below in Figure 1, cellulose is the primary polysaccharide in plant cell walls and vegetable fibers. Wood, some clothing, food and feed (grain, stalk, straw) all contain cellulose.

In 1977, Ratledge estimated that about 9<sup>11</sup> tonnes of cellulose were synthesized and replenished through photosynthesis annually. Fadel (1999) studied the status of selected by-products containing high quantities of cellulose and found that they represented almost two trillion Mcal of energy. The investigator also noted that the world's total by-products almost



equaled one billion tonnes of dry matter. Since 1999, quantities of by-products containing high amounts of cellulose and associated compounds have increased in developed and developing countries. In their review, Das and Singh (2004) noted that, "Cellulose is the most abundant material in the universe and makes up more than 50% of the total organic carbon in the biosphere and one third of the vegetation." When considering the use of all cellulosic compounds for fuel, it has been noted that globally, they represent 670 billion barrels of oil; this is 20 times the present worldwide oil consumption (Anon, 2011a).



1. Biology-blog, 2011.

2 Rose Laboratory, 2012.

3. Carbohydrate – cellulose, 2012.

**Figure 1.** Cellulose is an abundant material. Shown: (1) tomatoes; (2) tomato epidermis and cuticle – A. light micrograph of an unstained section; B. confocal optical section showing the cuticle and lipids stained orange and cellulose of the polysaccharide cell wall stained blue and (3) chemical structure of cellulose.

# 3. Cellulose - Fruit and vegetable production and loss

China, India and Brazil produce the most fruits in the world and the first two countries along with the United States produce the most vegetables (Tables 1 and 2, Freshel, 2012).

### 4. Loss of nutrients in agricultural by-products

Some cellulose is used for food, feed and fuel while a large quantity, along with valuable nutrients, is wasted as agricultural by-products. For the purposes of discussion in this chapter, fruit and vegetable by-products are defined as nutritious plant material not harvested and/ or wasted during and after production of food products (Figure 2).

Country	2008 (in T)	Average 03-07 (in T)	Var ø 03-07/08 (%)
China	184,560,585	160,912,654	14.70
India	53,133,000	40,034,665	26.40
Brazil	40,784,014	39,194,830	4.05
United States	30,404,237	30,420,653	-0.05
Italy	18,620,221	18,266,318	1.94
Iran*			
Turkey	18,418,811	17,503,291	5.23
Spain	17,518,300	18,072,322	-3.07
Mexico	17,105,610	16,307,257	4.90
Indonesia	12,647,246	14,814,412	-14.43
Other	228,723,846	242,734,060	5.77
Total	621,915,870	613,869,842	1.31
Total EU-27	65,910,712	67,858,847	2.87
% of EU share Of Total	10.60		

No data available

Freshel, 2012.

**Table 1.** Fruit production by country.

Country	2008 (in T)	Average 03-07 (in T)	Var ø 03-07/08 -13.53	
China	228,208,174	363,905,398		
India	48,868,700	60,985,290	-19.87	
United States	32,686,925	33,945,851	-3.71	
Turkey	20,818,183	19,870,895	4.77	
Russia	10,957,800	14,018,032	-21.83	
Spain	10,386,800	10,848,069	-4.25	
Italy	10,933,710	13,875,821	-21.20	
Egypt	10,926,895	13,926,043	-21.54	
Iran*				
Mexico	9.672,171	9,265,987	4.38	
Other	142,390,924	177,893,627	-19.96	
Total	525,850,281	627,300,111	-16.17	
Total EU-27	52,747,418	60,543,485	-12.88	
% of EU-share				
of Total	10.03			

No data available Freshel, 2012.

**Table 2.** Vegetable production by country



b. TED, Wageningen, 2012.

**Figure 2.** Fruit and vegetable by-products: a. waste, possibly from a home or restaurant and b. apples discarded during processing.

The nutrient composition of selected by-products has been evaluated (Chedly and Lee, 2001). More recently, Mirzaei-Aghsaghali and Maheri-Sis (2008) reviewed nutrients in several fruit and vegetable by products. Results for research at the University of California, Davis indicated that tomato pomace, a by-product from the processing of various types of

tomato products, contains 25.4  $\mu$ g/g of  $\alpha$ -tocopherol, 3.32  $\mu$ g/g of lycopene and 3.89  $\mu$ g/g of  $\beta$ -carotene (Assi and King, 2007). As shown in Table 3, other important nutrients in tomato pomace include crude protein, crude fat, vitamins and minerals.

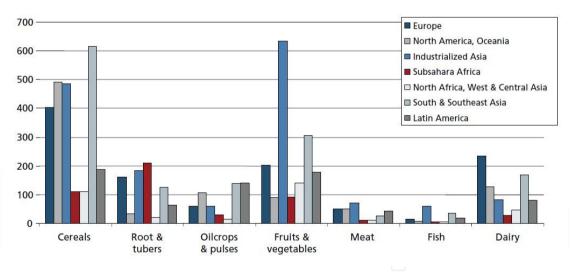
Analysis	Tomato pomace <sup>a</sup>		
AMEn	892.41 calories/lb (8.22MJ/kg)		
Protein	26.9%		
Crude fat	11.9%		
Crude fiber	26.3%		
Moisture	5.1%		
Ash	3.5%		

 $AME_n$ , gross energy of feed with corrections for gross energy of the excreta after correcting for retention of nitrogen by the body.

<sup>a</sup>King and Zeidler 2004, Assi and King 2008; Mansoouri et al, 2008.

**Table 3.** Selected nutrient content of tomato pomace.

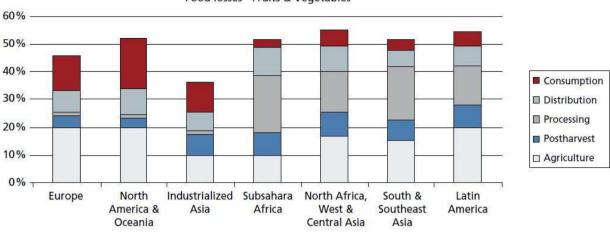
Figures 3 shows *production* of fruits and vegetables by areas of the world while Figure 4 shows percentages of fruit and vegetable *loss* along the food supply chain from production to consumption in many locations.



Bars for each commodity show countries as listed in the legion box (top to bottom) from left to right. Gustavasson et al. 2001.

Figure 3. Production volumes of each commodity group, per region (million tonnes).

Fehr and Romão (2001), using a 1998 report (discussing elevated food losses) by the Center for Packaging Technology (Centro de Tecnologia de Embalagem), estimated that for a typical Brazilian city of 440,000 people, the daily total loss of fruits and vegetables was 204 grams per person. This loss/person resulted in a total loss for the typical city of about 82 tonnes per day.



Food losses - Fruits & Vegetables

Percentage loss at various stages of the food supply chain in the bars is shown as listed in the legion box from top to bottom. Gustavasson et al. 2001.

**Figure 4.** Part of the initial production lost or wasted at different stages of the food supply chain for fruits and vegetables in different regions.

India, ranking second for worldwide production of fruits (10%) and vegetable (14%), can have loses as great as 30% to 100%, possibly due to climate, storage and handling conditions (Ashok, 1998; Gautam and Guleria, 2007). With Indian production of fruits and vegetables estimated at 150 million tonnes, the total waste could be 50 million tonnes per year (Gautam and Guleria, 2007).

According to Cuéllar and Webber (2010), in 1995, the United States Department of Agriculture reported waste for many agricultural commodities. Waste for products containing cellulose included grain (6.6 billion kg), raw and processed fruits (5.1 billion kg) and raw and processed vegetables (7.2 kg.). This loss amounted to 32.0%, 23.4% and 25.3%, respectively, of the food supply in each of the categories.

### 5. By-products in the environment

In many parts of the world, a majority of food waste clogs landfills and emits methane, a greenhouse gas in very rich to extremely poor countries. In addition to polluting the air, by-products can cause water pollution because it is difficult to burn materials with very high moisture content (Mirzaei-Aghsaghali and Maheri-Sis, 2008).

Although in the statement below, Apaak (2011) was referring to the transformation of byproducts to energy, the statement indicates how waste, especially that from fruits and vegetables, is discarded in municipal areas.

"A walk from Circle to the STC yard, a drive between the Achimota interchange and Lapaz, a visit to the vicinity of the landfill at Nungua, and a drive in sections of any of our regional capitals will prove that Ghana has a major waste management and disposal problem that can be a deterrent to economic development." Apaak, 2011.

# 6. Feed sustainability in the developing world – Use of fruit and vegetable by-products

In developed parts of the world, research is underway and technology is being used to convert fruit and vegetable waste to ethanol and remove coloring agents and antioxidants (Pap et al., 2004; Garcia-Salis et al.; 2010; Zhang, 2006; Yoshimurai 2012; Scalime Nutrition, 2012). Presently, the website for Scalime Nutrition promises one-stop shopping for determination of nutrients (especially polyphenols) from fruit and vegetable by-products and an analysis of the potential applications for nutrient use.

Certainly, in communities across the globe, some by-products will be composted or used for energy; however, in poverty stricken areas where protein consumption is limited, by-product use in the production of feed to produce more protein from small animals seems most urgent. For this discussion, these animals, easy to maintain in small numbers or large groups, include pigs, meat-type chicken, laying hens, turkeys and ducks. Presently, pig meat is the principal meat product consumed in the EU-27 (22.0 million tonnes in 2010) and in many parts of the world. Experts believe that consumption of chicken and turkey products will surpass that of pork in 2012 (Agricultural Products, 2011; Anon, 2011b).

Developing alternative feed sources for monogastric animals is especially important as traditional feed sources (especially corn and wheat) are converted into ethanol in the United States and other developed countries while greatly affecting the availability and price of these commodities in poor countries. Additionally, the cost of grain is expected to continue to rise in extremely food insecure countries due to consumption of traditional grain by humans, growing food needs of populations in emerging economies and harvesting problems associated with climate change (Yegani, 2007; FAO, 2011; Marcela and Ashitey, 2011).

"One billion people suffer from hunger every day. Yet the earth's population is expected to increase from seven billion in 2011 to more than nine billion people by 2050. The Food and Agriculture Organization of the United Nations predicts that food production will need to increase by 70% over the next four decades to meet anticipated demand. Part of the increase will be driven by higher demand for **animal protein** (emphasis added), especially in developing countries with rising incomes." (Strumbos and Derieux, 2011).

# 7. A case for alternative feed sources for production of poultry and eggs in food insecure areas

In many poverty stricken rural areas of the world, women and girls comprise the bulk of the agricultural workforce. They are actively involved in backyard poultry rearing due to benefits of providing meat and eggs for meals and supplemental income for their families. Backyard and small-scale meat-type chicken and layer production (the combination hereafter referred to as poultry production) continues even though there are many

constraints. Indeed, poultry production is promoted by humanitarian efforts to reverse poverty, increase food security and enhance nutrition/health of family members, especially children (ENAM, 2008). Some challenges affecting the success of nutrition programs are associated with education and communication structures. However, there is another challenge that is central to successful improvement of family nutrition by consumption of animal protein. The challenge is that many small scale farmers may not know how to produce alternative feed sources for poultry as the price of corn rises globally and becomes too costly for use. Indeed, lack of feed for small-scale production of poultry could have a significantly adverse effect on programs like ENAM.

Ghanaian poultry production, based on the use of corn and soy, makes a good case study. In their report, Ghana Poultry and Products Annual 2008, Flake and Ashitey highlighted the on-going condition for poultry production in the country. Due to government initiatives in the 1960's, the combined (small-, medium- and large-scale) poultry industry produced almost 95% of Ghana's chicken meat and eggs during the 1980's and 1990's. However, by 2001, a precipitous decline in production caused supply to slip to about 10 %. By 2008, low production was due to increased imports of cheaper poultry and the cost of grains, chiefly wheat bran or white and yellow corn. Whereas 50 kg of yellow corn cost \$24.70 in 2007, it rose to \$45.00 in 2008 due to the worldwide economic downturn. In 2008, feed cost represented up to 82% of variable production costs.

According to Marcela and Ashitey (2011), corn - a major staple food crop for the Ghanaian population – is presently produced in the country. Though data is sketchy, best estimates are that human consumption of all Ghanaian corn is at 85% while 15% is used to feed livestock with the greater quantity for poultry (~ 225,000 MT per year). From 2009 – 2011, the local wholesale price for 100 kg of white corn fluctuated between \$30.00 to \$34.00.

As Ghanaian corn production increases from an average annual supply of 1.5 million tonnes (Marcela and Ashitey, 2011) or remains steady, more of it may be substituted for malt in the growing brewing industry; ultimately less than 15% of the corn produced may be available at very high prices for the small poultry industry. As noted by the Food and Agricultural Organization (FAO), the International Fund for Agricultural Development and the World Food Program, "Food price volatility featuring high prices is likely to continue and possibly increase, making poor farmers, consumers and countries more vulnerable to poverty and food insecurity (FAO, 2011a)." If corn is not available for poultry feed during extremely stressful economic and environmental conditions, small-scale production certainly will not improve. It may not survive.

Flaherty et al. (2010) calculated that 1.5% of the Ghanaian higher education full time teaching equivalents were devoted to poultry research as compared to 93.1% for crops including cassava, cereals (maize, millet, and rice), cocoa, plantain, potatoes, oil palms, shea nuts and yams. If small family farmers in Ghana and other developing countries are to gain health and economic benefits from production of poultry meat and eggs, more research needs to focus on poultry production. In order to increase poultry production by small-scale farmers, research on alternative feed sources is needed.

Through international assistance in 2012/2013, Rwanda plans to develop Master's Degree programs in Animal and Plant Sciences at the National University of Rwanda (NUR) (USAID, 2012). A cooperative effort for experts in the Faculty of Agriculture at NUR to use fruit and vegetable by-products as well as that from other plant sources in developing feeding programs for mongastric animals would insure the availability of alternative feed sources for small-scale farmers in this country.

### 8. Challenges for use of alternative feed sources

As noted by Agudelo (2009), there are many challenges associated with use of alternative feedstuffs. These include anti-nutritional factors (disease or toxic factors), availability of raw material; composition and metabolizability of nutrients, consistency, cost (labor, processing, storage and transportation), health hazards, legality of use, palatability and overall effect of new feed on the resultant quality of the food. Each of these factors has to be considered and effectively minimized or totally eliminated when using alternative feed sources.

# 9. Endless possibilities for use of fruit and vegetable by-products in feeds

Two possibilities for inclusion of fruit and vegetable by-products in feedstuffs are discussed below.

#### 9.1. Bovine feedstuffs

Chedly and Lee (2001) proposed a number of tropical by-products (containing cellulose) that could be used as ingredients in alternative feed sources for pigs (Table 4). Additional by-products from Sub Suharan African countries for consideration include discarded cereals (maize, millet, and rice), cocoa, plantain, oil palms, shea nuts and other crops in rural and urban agricultural areas (agriculture on open urban lands) that are deposited as waste (In an email from P. Akowua, October, 2011; akowua963@yahoo.com). Researchers have tested fruit and vegetable waste from shops in Salamanca, Spain and to a market place in Medellin, Columbia for use in bovine feedstuffs (Garcia et al, 2005; Angula et al., 2012).

#### 9.2. Laying hen and poultry feedstuffs

High fiber agricultural products and by-products have been used as unconventional feedstuffs in non-feed-removal diets of laying hens. Diets containing 94% wheat middlings and various combinations such as 71% wheat middlings and 23% corn (Biggs et al., 2003, 2004) have been tested. Guar meal (10 and 15%) (Zimmerman et al., 1987), grape pomace with added thyroxin (Keshavarz and Quimby, 2002), various combinations of alfalfa (Landers et al., 2005; Donaldson 2005), and cottonseed meal (Davis et al., 2002) were also evaluated. Coconut meal and cumin seed meal were successfully used in layer and non-feed-removal molt diets in Asian and Middle Eastern countries (Ravindran and Blaire, 1992; Mansoori 2007).

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Feed	DM (%)	Per kg DM		Per kg Fresh Matter			Inclusion rate fresh	
		ME (MJ/kg)	CP (g/kg)	CF (g/kg)	ME (MJ/kg)	CP (g/kg)	CF (g/kg)	(kg/day)
Spent grain	22.0	8.2	260	130	1.8	57.2	28.6	5-20
Banana stems	9.5	5.5	20	210	0.52	1.9	20.0	5-10
Banana skin (ripe)	15.0	6.7	42	77	1.0	6.3	11.6	2-5
Rejected banana (ripe)	30.0	11.5	54	22	3.5	16.2	6.6	2-5
Cassava leaves	16.0	6.7	235	190	1.1	37.6	30.4	3-6
Cassava roots	28.5	12.5	16	52	3.6	4.6	14.8	5-15
Molasses	78.0	11.5	15	0.00	9.0	11.7	0	0.5-2
Breadfruit (ripe fruit)	29.8	10.8	57	49	3.2	17.0	14.6	4-8
Taro leaves	16.0	6.2	223	114	1.0	35.7	18.2	1-2
Taro Roots	25.0	13.2	45	20	3.3	11.25	5	2-5
Sweet potato (leaves)	12.0	5.8	200	145	0.7	24.0	17.4	10-20
Sweet potato (tuber)	30.0	13.5	70	25	4.1	21.0	7.5	5-10
Yam (leaves)	24.0	7.3	120	250	1.8	28.8	60	2-5
Yam (root)	34.0	13.5	80	25	4.6	27.2	8.5	2-5
Poultry litter	82	8.2	265	145	6.7	217.3	119.0	0.5-2
Olive cake	45.5	3.8	40	465	1.7	18.2	211.6	2-4
Olive leaves	56.8	5.7	105	300	3.2	59.6	170.4	3-6
Grape marc	37.1	4.9	138	410	1.8	51.2	152.1	1-3
Sugar-beet pulp	19.5	9.8	91	316	1.9	17.4	61.6	up to 20
Tomato pulp	22.5	8.0	215	350	1.8	48.4	78.8	up to 15
Wheat bran	89.1	8.1	160	137	7.3	142.6	122.1	1-3
Date palm fruit	87.6	12.0	32	50	10.5	28.0	43.8	0.5-1
Citrus pulp	23	10.3	75	200	2.4	17.3	46	up to 15

Key to columns: DM = dry matter. ME = metabolizable energy. CP = crude protein. CF = crude Chedly and Lee, 2001.

**Table 4.** Tropical by-products proposed for ensiling.

In California, the producer of close to 50% of the fruits, nuts and vegetables grown in the United States, the number of by-products that could be used in feed for poultry and other monogastric animals is quite abundant. Tomato pomace is one of these by-products.

Patwardhan and King (2010) reviewed types of non-feed withdrawal diets and suggested that tomato pomace as well as safflower meal, an abundant by-product from processing of safflower seed (Davies, 2002), could be used in molt diets. When comparing weight gain, feed consumption, and egg production, results revealed the usefulness of tomato pomace in non-feed-removal molt diets (Patwardhan et al., 2011a). Due to a decrease in bone density of hens fed tomato pomace, it was recommended that post molt diets of hens previously fed this alternative feed source contain an additional source of calcium (Patwardhan and King, 2011b). Research on the palatability of tomato pomace indicated that commercial feed pellets containing pomace (10%) and invert sugar (10%) could support adequate egg production (King and Griffin, 2012a, unpublished data) for a period of 14-days.

### 10. Production of tomatoes

A very popular food, eaten fresh or in many commercial products, tomatoes are produced in several regions of the world with China leading production followed by the United States where over 95% of the tomatoes are grown in California (USDA, 2012). Two developing countries - Egypt and India - are among the ten countries that produce 65% of processed tomatoes and concomitant tomato pomace (Branthôme, 2010). Tunisia (engaged in recent tomato wars over the differential in prices between fresh tomatoes and those used for processing) produces processed tomatoes in Africa and the Mediterranean (Republic of Tunisia, 2011). Nigeria is ready for increased tomato production and processing also (IITA, 2011). Additionally, tomato production and processing is underway in EU countries, African countries, Southeast Asian countries (Vietnam, Cambodia, and Laos), Mexico, Central America and South America (Branthôme, 2010; AVRDC, 2011).

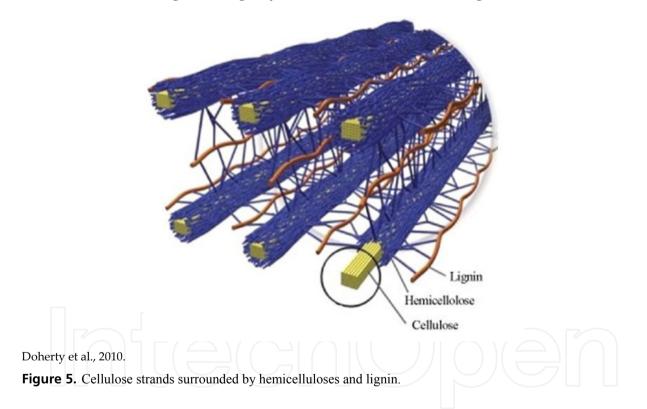
Tomato pomace - a mixture of cores, culls, trimmings, seeds, peels, liquor and unprocessed green tomatoes remaining after processing - is not in constant high demand for use in feed products in developed countries (Patwardhan et al., 2010) or developing ones (In an email from P. Akowua, October, 2011; akowua963@yahoo.com Akowua, 2011). It is usually treated as waste and discarded. Wet tomato pomace, combined with wheat, may be fed to ruminants to enhance their growth performance (Denek and Can, 2006; Abdollahzadeh, 2010). Holistic Natural Pet Food (2010) reported that tomato pomace improved the palatability of cat food.

# 11. A major deterrent to the increased use of tomato pomace and other by-products of plant origin

Tomato pomace is a high protein and high fiber by-product (Table 3). Furthermore, it contains  $\alpha$ -tocopherol (Vitamin E, an antioxidant) and lycopene (an antioxidant and a coloring agent) that could be additional feed ingredients for poultry (King and Zeidler, 2004;

Al-Betawi, 2005; Assi and King, 2008). However, use of this by-product and many others that could be added to feed of monogastric animals is limited due to high fiber content (Assi and King, 2007, 2008; Patwardhan et al., 2011a, b). As with many by-products, the major fiber components - cellulose, hemicellulose and lignin - of tomato pomace are difficult to digest by non-ruminant animals like poultry because the greatly dispersed physical and chemical structure created by lignin polymers and their cross linking with other polysaccharides prevent free enzymatic access to cellulose and hemicelluloses (Figure 4).

Structurally, cellulose microfibrils are ordered polymer chains of  $\beta$ -1,4 linked D-glucose units with tightly packed crystalline regions (Figures 5). A diverse group of carbohydrates that are referred to as hemicelluloses, are soluble in strong alkali solutions, linear (no microfibril formation) and flat, with a  $\beta$ -1,4 backbone. Having relatively short side chains, hemicellulose, known as cross-linking glycans, form hydrogen bonds with cellulose (Saupe, 2011, Figure 5). Lignin, a cross-linked macromolecule composed of phenylpropanoid monomers, adds strength and rigidity to cell walls (Smook, 2002, Figure 5).



# 12. Selected methods for treatment of lignocellulosics

Partial or total elimination of cellulose and accompanying polysaccharides in fruit and vegetable by-products is paramount for digestion by monogastric animals. Moreover, retention of nutrients for incorporation into feed is equally important. Polygastric animals can consume high fiber by-products which are often good sources of protein. However, consumption of these by-products by monograstric animals can result in diarrhea and ultimately weight loss. Ensiling, enzymatic conversion and use of calcium hydroxide under various condition are a few of the approaches discussed below.

#### 12.1. Ensiling

Ensiling is generally used to store excess grain that might otherwise deteriorate over time. During ensiling, some bacteria secrete enzymes to degrade cellulose and hemicellulose to various simple sugars while others reduce simple sugars to acetic, lactic and butyric acids, causing a low pH that helps to preserve surplus grains. High lignin vegetable and fruit by-products, with or without ensiling, can be degraded to various nutrients by rumen herbivores (cattle, sheep, goat and deer) through digestion. These by-products cannot be included at high concentration in diets of monogastric animals like poultry. Therefore, probably, this treatment could only be used for low lignin ( $\leq 5\%$ ) by-products. Chedly and Lee (2001) proposed a list of by-products that could be ensiled or otherwise treated (Table 3). Another deterrent to ensiling is that when a specific ensiled by-product has been analyzed, the resultant material is usually lacking key vitamins, minerals or amino acids (Ly, 2006).

#### 12.2. Enzymatic conversion by organisms

Bioconversion by enzymatic reactions during solid state fermentation by several organisms has been studied (Joshi and Sandhu, 1996; King and Assi, 2007, 2008, 2009; Demers et al, 2009). When comparing untreated and treated samples, Joshi and Sandhu (1996) found that after removal of ethanol, dried apple pomace had more protein, fat, vitamin C, zinc, manganese, copper and iron after solid state fermentation with *Saccharomyces cerivisae*, *Candida utilis, and Torula utilis*. Other components remained unchanged.

*Pleurotus ostreatus* (Assi and King, 2004, 2007, 2008) was used to convert tomato pomace amended without and with 487µM manganese/g substrate. In treated substrate without manganese, cellulose and hemicellulose were reduced. However, the mineral activator inhibited fungal growth and did not degrade lignin. An investigation was undertaken to determine if the O<sub>2</sub> consumption rate and CO<sub>2</sub> evolution rate of *P. ostreatus* were affected by manganese. Results indicated no delay for peak and cumulative CO<sub>2</sub> rates; thus, manganese may have reduced the metabolic activity of *P. ostreatus*. For future studies, researchers suggested use of (1) lower levels of manganese and (2) *P. ostreatus* in an O<sub>2</sub> atmosphere of > 20%.

#### **12.3.** Enzymatic hydrolysis by commercial enzymes

Enzymatic hydrolysis is not as quick as chemical catalytic hydrolysis; however, it (1) requires less time in the reactor, (2) can be performed at much lower temperatures which may be needed to conserve ingredients in feedstuffs, (3) has lower utility costs and (4) allows for a higher conversion rate than acid hydrolysis which is more equilibrium driven and can produce many end products (Purwadi, 2004; Purwadi et al., 2006; Demers et al, 2009). Although their main application was for production of biofuels rather than the release of valuable components for use in animal feeds, Demers et al. (2009) chose enzymatic hydrolysis to investigate the break down of available cellulose in apple pomace, wood

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shavings and swithchgrass feedstocks. Other investigators refined the use of enzymatic hydrolysis as well by utilization of the cellulose enzyme complex of *Tricoderma viride* (Lindsey Wilke, 2011) and by the use of an immobilized  $\beta$ -glucosidase reactor (Issacs and Wilkes, 2011).

Work on enzymatic hydrolysis has been enhanced by use of catalysts needed to accelerate enzymatic activity. In their work on "cellulose enhancing factors" for oxidative degradation of biomass component, Quinlan et al. (2011) revealed that (1) GH61 enzymes need copper for maximal activity (2) that ascorbate and gallate (small molecule redox-active cofactors) enhance development of cellodextrin and oxidized products by GH61 and (3) methylated histidine was located in the coordination sphere of type II copper in the enzyme's active site. While most studies are focused on the final production of ethanol, hopefully, some research will focus on controlling enzymatic breakdown of cellulose in order to preserve protein and other nutrients for feed inclusion.

#### 12.4. Heating and calcium hydroxide treatment

Various methods for solid state bioconversion and enzymatic conversion are proposed often for the ultimate breakdown of lignocellulosics to ethanol. However, nutrients needed for feed inclusion will be lost and even if modified when further developed, cost will prohibit their use by small-scale farmers in developing countries. Combinations of heat, treatment with calcium hydroxide and other methods to remove varying quantities of fiber may be more feasible for small- and large-scale use of by-products for feed ingredients.

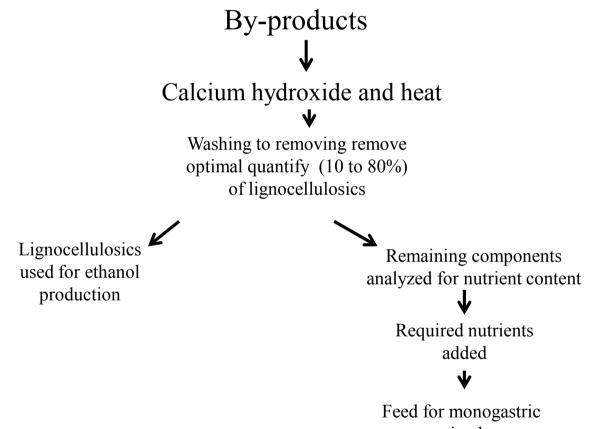
Research has been conducted on the use of heat to reduce lignocellulosics in by-products (Squires, 1992; Carmen, 2003; Al-Betawi, 2005; Mondragon et al., 2010; King et al., 2012). According to Carmen (2003) and Mondragon et al. (2010), alkalinity and heat used during nixtamalization improved the availability of some nutrients in corn while reducing the amount of others. King and Griffin (2012b) are continuing studies that reduce cellulose, hemicelluloses and lignin after using combinations of heat (autoclave and microwave) and calcium hydroxide, which is less caustic than sodium hydroxide.

Calcium hydroxide (or inexpensive slaked lime) is available in many parts of the world (Goats, 2010). Where it is not readily available, other methods of producing it are possible. In communities near oceans, ash containing calcium hydroxide can be produced from burning pulverized oyster shell (without lead) or egg shells. In geographical zones away from the ocean, egg shells can be a source of calcium hydroxide.

In a recent study, Janker-Obermeier et al. (2012) found that at least 4–5 wt% of NaOH (~ 0.4 g/g biomass) was needed to dissolve hemicellulose (xylan) from straw at high amounts (73%) in the supernatant. Moreover, solubility of lignin was increased by microwave energy (between 120 and 735 J/g dry mass). In solid wheat straw, excessive amounts of hemicelluloses (>80%) and lignin (90%) could be removed without inordinate degradation of saccharide or solubilization of cellulose. These recent results suggest that, eventually, mild treatments for removal of lignocellulosics from fruit and vegetable by-products will be

developed so that remaining nutrients can be incorporated into feed for monogastric animals.

As noted above, during processing of fruit and vegetable by-products for monogastric animals, some fiber and, ideally, all of the nutrients should be maintained. A schematic for processing of fruit and vegetable for this purpose is proposed (Figure 6). As mentioned above, procedures that combine various amounts of heat (no heat, autoclave and microwave) and calcium hydroxide are being investigated (King and Griffin, 2012). While cellulose in fruit and vegetable by-products could be used as fuel, potentially, it, along with associated nutrients, could be used as feed ingredients in the diets of animals. Procedures that maximize the amount of ligninocellulosics that can be removed while minimizing nutrient loss should be investigated thoroughly. Once fully developed these procedures, characterized by low technology input, can be made available to small-scale farmers in developed and developing countries where enzymatic reduction of high fiber content in byproducts may not be a viable alternative.



animals

Figure 6. Removal of lignocellulosics from fruit and vegetable by-products.

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