

Grain: Composition and Functionality.

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Introduction: It is not my intent to provide a definitive source covering every aspect of grain use in brewing. Rather, I want to open the door on many of the contributions cereal grains make to our beer and, hopefully, foster a desire for and provide a first step toward independent research on the part of the reader. A number of references are given and all are available on the World Wide Web.

We should all be familiar with the two chief components of grain: starch and protein. But what are these components really and what do they do to my beer? We have all heard terms like “Protein Haze” thrown around but does this protein stuff form a haze just because I have too much of it in my final beer or are there other factors to consider? We convert starch into simple sugars (glucose, maltose, and maltotrios) during the mash. This is pretty simple and straightforward, isn't it? There's really nothing else about starch I need to know, is there? What else is in grain that has an impact on the final product? Hopefully this article will answer some of these questions and raise a whole host of new questions you had not previously considered.

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On with the show....

Proximate Percentile Composition of Cereal Grains (adapted from Haard et al., 1999).

| Component | Wheat | Rice | Rye | Oats | Maize | Barley | Millet | Sorghum |
|----------------|----------|---------|------|-------|-------|--------|--------|---------|
| 1. Starch | 60-68% | 64% | 72% | 63% | 64% | 56% | 63% | 63% |
| 2. Water | 8-18% | Typical | | | | | | |
| 3. Protein | 7-18% | 7.3% | 8.7% | 9.3% | 9.8% | 11.0% | 11.5% | 8.3% |
| 4. Pentosans | 6.2-8% | Typical | | | | | | |
| 5. Ashes | 1.5-2% | 1.4% | 1.8% | 2.3% | 1.4% | 1.9% | 1.5% | 2.6% |
| 6. Fats/Lipids | 1.5-2% | 2.2% | 8.7% | 6-10% | 4.9% | 3.4% | 4.7% | 3.9% |
| 7. Cellulose | 1.0-5% | 0.8% | 2.2% | 2.3% | 2.0% | 3.7% | 1.5% | 4.1% |
| 8. Maltose | 0.6-4.3% | Typical | | | | | | |

Starch is a hard granular carbohydrate composed of glucose polymers. There are two starch fractions:

Amylose is a straight chain glucose polymer that can be almost completely hydrated by alpha and beta-amylase. Native starch is 20-30% amylose (Native meaning: unmolested, unaltered grain as found in nature).

Amylopectin is a more complex, branched, three-dimensional lattice structure which is less soluble than amylose and is not completely hydrated by beta- or alpha-amylase. Typically composed of an Anhydric Core, 2 Arene Aldehyde Ocsion molecules, and 6 Ethyl Ester molecules (I use this description only to introduce the idea of esters being bound in molecules found in grain and that this is one source of esters in the final beer product. This subject will be visited in some detail later). Amylopectin will not dissolve in cold water and dissolves in very hot running water only after 12 hours exposure. 70-80% of native starch.

Amylose/Amylopectin ratios of various sources (data from numerous sources).

| Grain | Amylose | Amylopectin |
|--------|---------|-------------|
| Barley | 25% | 75% |

| | | | |
|---------------------------------------|--------|--------|----------------|
| Corn | 28% | 72% | |
| Potato | 21% | 79% | |
| Rice (Normal: Long, Basmati, Jasmine) | 25-30% | 70-75% | (Cooks Dry) |
| Rice (Waxy: Pearl, Medium, Brown) | 16-22% | 78-84% | (Cooks Sticky) |
| Tapioca (Cassava) | 15-18% | 82-85% | |
| Wheat | 25% | 75% | |

Essentially, amylose, because of its linear structure, bonds when heat is applied in the presence of water resulting in stickiness (gel strength). On the other hand amylopectin, because it takes on a more complex 3-dimensional helix structure akin to DNA, tends to incorporate more water and results in greater viscosity.

Amylopectin rich potatoes and tapioca have been used by some brewers to provide additional “silkeness” to their brews. There are two basic potato types: bakers and boilers. Bakes such as Russet or Idaho are high in amylose. Boilers such as Red or White Crescent are high in amylopectin; choose these varieties to add additional silkeness to your potato beer. Amylopectin rich tapioca should provide a similar result.

As a construct for understanding, starch can be seen as functionally progressing through three distinct utilization phases. When native starch is associated with water it swells slightly but acts as an amorphous solid, some water remains unbound, and starch granules can settle out of solution over time. About 1 gram of water is associated with each gram of starch. When heat and pressure increase, molecular motion of starch chains increase. The second phase, gelatinization, occurs at a certain temperature threshold. 5-30 grams of water per gram of starch are now associated.

For a molecule of starch to become readily available for hydrolyzation and eventual fermentation, it must be gelatinized. Amylose is more readily soluble (gelatinized) than amylopectin. Solubility is a function of water available, heat, and pressure-shear. Pressure is just that, static pressure, PSI. Shear is the range of dynamic forces in the boil as well as mechanical manipulation (stirring).

Percent of Amylose and Amylopectin Dispersed and Soluble in Water (R.D. Waniska, 199?).

| <u>Process</u> | <u>Amylose</u> | <u>Amylopectin</u> | <u>Granule</u> |
|---|----------------|--------------------|----------------|
| Initial (excess water) | less than 5% | less than 2% | Rigid |
| +Time+Temp (to boiling) | 30-40% | less than 10% | Rigid |
| +Time+Temp+Pressure-Shear (Rolling Boil) | 40-50% | 10-50% | Deformed |
| +Time+Temp+++Pressure-Shear (Pressure Cook) | 50-60% | 10-90% | Deflated |

As can be seen, short-chain amylose is fairly readily available from a short boil. However, because amylose is entirely hydrated by beta-amylase, a short boil negates the purpose of using body-enhancing adjuncts in our beer. You would, in affect, merely be adding more stuff to turn into simple sugars. Because amylopectin is a complex starch not completely converted by either of the chief diastatic enzymes, it is this molecule (in addition to other even less soluble fractions, mostly proteins) we are after when using adjuncts to increase body as with unmalted barley in a stout or to improve mouthfeel when using oats in a stout. What is left after amylase is done tearing apart amylopectin is beta-limit-dextrin. This is what we are after when using body-increasing adjuncts.

While fractions other than beta-limit-dextrin may play a more important role in mouthfeel, as long as you are using unmalted adjuncts for mouthfeel, you may as well get all you can out of them. For this reason, a minimum 30-minute rolling boil should be used to gelatinize adjuncts that are being employed to increase body or mouthfeel. Shear forces need not only be thought of as being supplied by a hard boil. The importance of mechanical shear as a result of stirring cannot be overemphasized. Pressure cooking adjuncts is also an excellent way to ensure thorough gelatinization. Rice and maize gelatinize at temperatures 10-20°C higher than wheat, rye, and oats. This should be considered when gelatinizing rice and maize.

The third utilization phase is retrogradation. When heat and pressure-shear are no longer being applied, retrogradation begins to happen almost immediately after the temperature drops below 115°C. At this tem-

perature, amylose quickly begins to form an aggregate gel network which traps amylopectin. These bonds become very stable at temperatures below 50°C. In typical amylose/amylopectin gels, retrogradation results in the formation of amylose rich partially crystalline polymer systems that are enzyme resistant (Enzyme Resistant Starch, RS). Crystallinity of RS fractions increases over storage time of the gel (R. Eerlingen, 1994). For these reasons, gelatinized adjuncts should be introduced directly to an enzyme rich environment (the mash) immediately upon completion of conversion. (Depending on beer style, I often use the boiling hot adjuncts to step up from one rest to the next; from acid/gum rest to a protein rest for example.)

Water is found in all grains. Moisture content of 13% is acceptable for grain storage of six months or less but should be 12% or below for long term storage. Malt should be even dryer; typically below 6% for storage.

At the end of a one to two day steeping period, barley malt typically contains between 42 to 48 percent moisture. This begins to break down water-soluble fractions. At the same time, arabinosidase is the first enzyme activated starting germination. Arabinosidase is one of several hemicellulose enzymes which break down cell walls. Next proteolytic enzymes go to work hydrolyzing proteins. Finally diastatic enzymes become active in the nearly fully modified kernel. Malt is then kilned as desired to prepare the malt for storage. At the end of the kilning process, moisture content is roughly 2 to 5.5% in most commercially produced base malts. Bone dry malt ensures enzymatic stabilization and long shelf life.

Crystal malt is kilned quite moist to allow starch conversion in the kernel. Crystal malts tend to be 96-98% sugar upon completion. Lightly colored crystals (<20L) tend to have moisture content near 7% or above. With high sugar content and relatively high moisture, light crystal malts such as Hugh Baird Light Carastan_{im} should not sit around for years prior to use.

Proteins are made up of amino acids. Naturally occurring amino acids are formed by an amino- (-NH₂) and a carboxyl (-COOH) both attached to the same carbon atom. There are four proteins typical to all cereal grains: Albumins, Globulins, Prolamines, and Glutenins. Albumins are soluble in water; the other three are not. (Solubility is important in that, the less soluble something is, the harder it is to get the stuff into your wort. If it doesn't go into solution, it more than likely gets left behind in spent grain or trub rather than becoming part of the final beer.)

Distribution of Proteins in various Cereal Grains (Haard et al., 1999).

| Grain | Albumin | Globulin | Prolamine | Glutelin | |
|---------|---------|----------|-----------|----------|--------|
| Wheat | 9-15% | | 6-7% | 33-45% | 40-46% |
| Rye | 10-44 | | 10-19 | 21-42 | 25-40 |
| Barley | 12 | | 8-12 | 25-52 | 52-55 |
| Oats | 10-20 | | 12-55 | 12-14 | 23-54 |
| Rice | 5-11 | | 10 | 2-7 | 77-88 |
| Sorghum | 4 | | 9 | 48 | 37 |
| Maize | 4-8 | | 3-4 | 47-55 | 38-45 |

Albumin is a water soluble protein which coagulates upon heating (forms a majority of the hot break). It is hydrolyzed to peptides and amino acids by proteolytic enzymes. Albumin is common to grains, eggs, milk, and blood plasma (not to be confused with the technical term for egg white, albumin, which is comprised of ten separate proteins. Egg white is 53% albumin protein). Egg white, because of the binding characteris-

tics of some protein fractions, has been used in the past as a fining agent. Albumin is known to bind flavinoids. Albumin is capable of generating a foam and providing a degree of stability, as in egg whites and milk. Beer foam is 90% carbohydrates and only 10% protein. Albumin fractions responsible for foam stability are known as Amphiphilic Proteins, meaning one end of the molecule is hydrophilic while the other end is hydrophobic, as is the case with soap molecules.

The primary albumin derived amphiphilic protein responsible for foam stability is Lipid Transfer Protein 1 (LTP1). LTP1 has been shown to concentrate in beer foam. Native LTP1 has been shown to have poor foam properties however (Sorensen et al., 1993). It is only after enzymatic fragmentation and denaturation which occurs in wort boiling that the protein becomes a foam promoting agent (Marion & Douliez, 1999). Excessive proteolysis results in a diminution of foam stability however (Kapp & Bamforth, 2001). This proteolysis can take place as a result of excessive exposure to endogenous grain proteases during the mashing process. It can also occur while sitting on the trub for too long or after bottling, as a result of an enzyme excreted by dying yeast cells known as Protease A (Kogin et al, 1999). Many people who cellar “real” beer for years are familiar with this phenomenon. Often foam just isn’t as robust as it was when the beer was “fresh.”

Two other amphiphilic albumin fractions are Protein Z and the (puro-) Indolines (a & b). Both enhance foam stability. Of particular interest are the puroindolines apparent increase in foam stability in the presence of some lipids (Marion & Douliez, 1999). Incidentally, silica hydrogel, chillproofing enzyme, and tannic acid adsorb, denature, or bind both protein Z and LTP1. While these stabilizing agents primary role is to remove hordein derived haze components, they also remove 3 to 6% of Protein Z and 4 to 16% of LTP1 (Sheehan et al., 1999). The trade off for clarity using these agents is a marginal reduction in foam stability.

Globulin describes any of a large family of proteins which are spherical or globular in shape and are found throughout the plant and animal kingdoms. For conceptualization purposes, think of immunoglobulins which are the antibodies of the immune system. Globulins bind and transport a variety of substances including lipids, hormones, and inorganic ions. Globulin is soluble in weak salt solutions and can be a component of haze; they precipitate out of solution at temperatures below 170°C. Their most important function in the boil is to bind polyphenols and remove them through precipitation during the cold break; 15-25% of Globulin and Prolamine are lost to protein-polyphenol complexes in the cold break and make up 20-30% of cold trub (Barchet, 1994). Albumin and Globulin derived polypeptides are chief foam forming agents and are little changed from their native state by the malting process. These proteins have a relatively high amino acid content in well-balanced proportions. They are completely transferred to aqueous solution during the mash process producing a good medium for yeast growth (Packa et al., 2003).

Prolamines are a group of globular proteins high in glutamic acid and proline, a non-essential amino acid. Prolamines found in various grains are: gliadin in wheat, secalin in rye, hordein in barley (four types B, C, D & ?), avenin in oats, and zein in maize. These are the storage proteins found in the grain germ and are those proteins most affected by the malting process. They are soluble only in alcohol solutions of greater than 70% strength.

In readily modified commercial malts, typically about 50% of the hordein fraction passes into the wort. Hordein appears to be a major contributor of Free Amino Nitrogen (FAN) in the wort. FAN and solublized proteins appear to be dictated in part by hordein levels in the native barley. Maltsters accept barley which is between 9% and 11% protein. Because the amount of hordein in the barley controls the ease with which protein is converted, it’s important to ensure the source barley remain within this narrow band. While nitrogen fertilizer has some effect on protein content (up to 5% change in content), the time at which the barley is sown has a more profound effect. Early (May) sowing results in lower protein content than late sowing (July) (Osman et al., 2001). Do not make the assumption however that this means “Hard Red Winter Wheat” is really low in protein (gliadin) because it grows in the very early spring. “Hard” wheat varieties, regardless of the time of year in which they are sown, are high in protein relative to “soft” varieties.

The vast majority of chill haze experienced in commercial beers is comprised overwhelmingly of hordeins and are relatively rich in **Proline** (Robinson et al., 2001). Proline complexes with polyphenols, mostly tannins, to form chill haze. Albumin and globulin derived polypeptides can also be responsible for chill haze but they come out of solution only after hordein derived species. Indeed, foam forming polypeptides (Al-

bumin and Globulin derived) come out of solution as haze with a commensurate decay in foaming ability of beer over time (Bamforth, 1999). As with most proteins, Proline is an isomer, that is, it can exist in two different shapes yet retain the same molecular structure. The folding or Cis-Trans Isomerization of proline can be enzyme or temperature induced. Proline being the chief culprit in chill haze, a study of its percentile quantity in various grains should be informative:

Partial amino acid composition (mole percent) of Prolamine fraction of various grains (Haard et al., 1999).

| Amino Acid | Wheat | Rye | Barley | Oats | Rice | Maize |
|----------------|-------|-----|--------|------|------|-------|
| Glutamine | 38% | 36 | 36 | 35 | 20 | 20 |
| Proline | 17 | 19 | 23 | 10 | 5 | 10 |
| Glycine | 3 | 5 | 2 | 3 | 6 | 3 |
| Cysteine | 2 | 2 | 2 | 3 | 1 | 1 |
| Lysine | 1 | 1 | 1 | 1 | 1 | Trace |

As can be seen from the foregoing table, Prolamine derived proline quantities in wheat, rye, and barley are 1.7-4.6 times greater than quantities found in oats, rice, and maize. The ratios are similar for glutelin derived proline as well. It is generally accepted that replacement of barley malt with a percentage of rice or maize will dilute all types of haze forming precursors, while wheat, rye, and barley adjuncts will increase the risk. This seems to lend some face validity to the proline-causes-chill-haze assertion.

Note that while gliadin (wheat prolamine) and glutelin combine to form sticky gluten in wheat, rice is essentially devoid of gluten because of very low prolamine levels despite high glutelin levels. Rule of thumb: if it forms a sticky glutinous dough like barley and wheat, you have gluten (bad for gluten intolerant people) which means prolamine derived proline which means greater potential for chill haze.

As an interesting aside, the astringency of polyphenols, specifically tannoids, results from their combination with and precipitation of salivary proline-rich proteins (PRP's), which reduces lubrication in the mouth. The tannins are, in effect, tanning the proline, just as they would leather. Weak acids, as in beer, enhance this effect. This is a tactile sensation perceived by the trigeminal nerve rather than a taste (Siebert & Chassy, 2002).

Glutelins are a group of simple storage proteins making up roughly 40-55% of protein found in brewing grains. They are second in the order of breakdown after prolamine in the malting process. The fraction that remains after extraction of the grain with water, salt solution, and alcohol is glutelin; in other words, it's tough stuff. While glutelin is insoluble in neutral solvents it is readily soluble in dilute acids or alkalis. I have read seemingly conflicting reports with regard to the role glutelin plays in brewing. One article states, "Glutelins do not pass to the wort" (Packa et al., 2003). On the opposite end of the continuum: "Among the native barley protein substrates, glutelins were hydrolyzed most effectively...by endoproteases," (Osman et al., 1999). The consensus opinion appears to be that glutelins are degraded more extensively than other protein fractions during the malting process and, as a result, appear to be a major contributor of Free Amino Nitrogen (FAN) to wort. It is not clear if glutelin has any major impact on the mashing process or the quality of beer other than this contribution.

Proteolytic Enzymes are found and act both on the inside of the grain (endogenous) and on the grain husk (exogenous). The great majority of endogenous enzymes are not present in the ungerminated barley but form during the germination process. There are over 40 endoprotease activities which have been identified. In the malting process, these proteases are most active on the third day after steeping (Jones, 1999). It has been generally accepted that protein degradation due to proteolytic activity occurs during the malting process and that proteolytic activity is minimal during the mashing process due to inactivation of the proteases during kilning. This is not the case however (Osman et al., 1999). Barley malt samples removed at various stages of the typical American malt kilning process showed no protease degradation up to the 85°C step and only partial denaturing of some proteases at higher temperatures. Further, of the soluble protein found in wort, 43% is preformed in the barley grain, 32% is solublized in the malting process, and 25% is released

during mashing (Jones, 1999). Clearly, proteolytic activity should be expected during the mashing process when using light colored fully modified base malts, and especially green and undermodified malts.

Some proteases are heat sensitive while others are relatively heat stable. Those which are heat stable (surviving 65°C for greater than 40 minutes) are most active on glutelin, globulin, and prolamine, in that order. Optimal conversion temperatures in the mash in degrees Celsius are: prolamine-40, glutelin-50, and globulin-60 (Osman et al., 1999). Note 50°C corresponds with the traditional 122°F protein rest. Above 60°C in the mash, all protease activity is quickly extinguished with virtually no activity remaining after 10 minutes at 70°C. Note the same enzymes are denatured more readily in solution (mash) than in the kernel (malting/kilning). Proteases, and presumably other enzymes, are protected in the kernel.

Endoproteases are broken into four classes: Cysteine, Serine, Aspartic, and Metallo-. Cysteine is a sulfur containing crystalline amino acid which is responsible for 90% of proteolysis in germinating barley (Kihara et al., 2002) and the majority of proteolysis in the mash. This class breaks down the gum that forms on top of some single-step infusion mashes (Mikola, 2001). While active during the malting process, serine class enzymes do not appear to play a significant role in the mash (not thermal stable, denatured during kilning). Aspartic and metalloproteases are amino acids which play a role during the mash process. The aspartic class of enzymes account for nearly all of the endoproteolytic activity in unmalted barley (Jones, 1999). Metalloproteases are a very powerful class of enzymes which include various fungal secretions as in *Streptomyces* sp. and are also found in Rattlesnake venom

Pentosans, beta-glucan, proteins, and phenolic acid are components of the cell walls of the starchy endosperm. Endosperm cell walls are most properly called hemicellulose and make up about 6.2-8% of the grist. Because the majority of the material in hemicellulose is pentose sugar, it is convenient to refer to the bulk cell wall product as pentosan. During the malting process, it is necessary to first break down the cell walls before starch can be released.

Pentosans are a group of polysaccharides found with cellulose and which yield pentoses when hydrolyzed. Pentoses are sugars having five carbon molecules and are generally unfermentable by *Saccharomyces* sp. This group includes Ribose (which is found in every living cell, RNA, DNA), Arabinose, and Xylose. About 50% of pentosans are water-soluble. Those which are not readily soluble, are acted on by a hemicellulase enzyme complex during germination. Hard wheat varieties have 150% greater amyloplast derived pentosan content than soft varieties (Bettge & Morris, 1997). While comprising only 2.5% of wheat flour, pentosans are responsible for 23% of water that is bound in bread dough. Because pentose sugars are largely unfermentable and form a gum by binding nearly ten times their weight in water, they contribute to body and mouthfeel. This contribution to body and mouthfeel has been largely under-appreciated up to this point and should be more greatly explored by the brewing community.

Arabinoxylan is the major contributor of pentosans and is found in all grains. While malt culms (dried rootlets) are mostly protein, they are also high in arabinoxylan. This may be one reason why green home malted wheat results in a more substantial mouthfeel than commercially malted wheat. This polysaccharide is made of two pentose sugars: arabinose and xylose. Arabinoxylan is water-soluble. It is also acted on by arabinosidase, xylobiase, and endo- and exo-xylanase. These enzymes are present in the native kernel and are enhanced during germination. Arabinosidase is the first detectable hydrolysis product in steeping grain. It appears that arabinosidase must act first before hydrolysis of the xylan main chain, beta-glucan, and other cell wall structures can occur. However, the enzymes responsible for complete arabinoxylan degradation are not produced until late in the germination process. For this reason, high levels of this polysaccharide may survive through the brewing process into the beer (Lee et al., 1999). Again, arabinoxylan and its derivatives are pentose sugars which are gummy in solution. They increase body, mouthfeel, and appear to increase surface tension acting as a foam stabilizer. Most enzymes have limit enzymes which control their activity. Unmalted wheat appears to have a limit enzyme (*Triticum Aestivum* Xylanase Inhibitor, TAXI) responsible for inhibiting xylanases in barley. In Weizenbier and Witbier, prodigious use of unmalted wheat leads to the particular mouthfeel and body characteristic of those beers. TAXI appears to be at least partially responsible (Winok, 1999).

Because it is not broken out separately in the first table and is a cell wall constituent similar to arabinoxylan, we will discuss **beta-glucan** here. Beta-glucan is the predominant part of endosperm cell walls in bar-

ley, oats, and to a lesser extent, wheat, and is made entirely of simple glucose units. It is also a main component of yeast cell walls. Its water binding capacity is similar to pentosans; it forms a gum. When doctors tell you to get more soluble fiber in your diet, beta-glucans are what they are talking about; arabinoxylan appears to have some of the same characteristics. Because beta-glucanases are quite active between 98-113°f, this range may be used as a gum breaking rest if no proteolytic activity is desired. There is one exogenous and three endogenous beta-glucanases which, in addition to the arabinoxylanases listed previously, make up just a few of the hemicellulose complex or gum breaking enzymes.

Ash is the residue which remains after all organic matter of the grain has been incinerated. It consists of mineral matter and serves as a measure of the inorganic salts that were in the original grain. On a dietary note, of the commonly consumed cereal grains, oats have the greatest ash/mineral content with some varieties running up to 5%. These minerals, in addition to those found in brewing water, are important to yeast nutrition.

Fats and Lipids are typically considered bad for foam; their derivatives can be major flavor and aroma contributors however. For ease of understanding in the brewing context, both fats and lipids are made up of fatty acids and are generally not soluble in water but are soluble in acids, alcohol, bile...and will be referred to simply as lipids. Lipids are hydrophobic, as are the large glycoproteins (molecules made of both protein and carbohydrate, the albumin and globulin polypeptides discussed earlier combine with carbohydrates to form glycoproteins) that make up foam, and compete for position on the bubble surface reducing the ability of the proteins to entrain liquid (Bohnsack et al., 2003). Lipases, the enzymes which hydrolyse fats and lipids, are most active during the fourth day of germination liberating free fatty acids. Lipase, specifically Triglycerol Acylhydrolase, catalyzes the hydrolysis of triglycerides into free fatty acids (FFA) during malting and in the mash. Kilning appears to reduce lipase activity by only 10% with activity in the mash remaining stable between 107°f and 153°f. Lipase activity was found to decline by about 40% when the temperature in the mash was held at 165°f for 20 minutes (Stanley et al., 2001).

Three main lipid fractions find their way into beer: neutral lipids, free fatty acids, and phospholipids. The first two deteriorate foam stability proportional to the amount present. Phospholipids appear to have no foam destabilization effect however (M. Hollemans et al., 1991).

Esters and Amides are naturally occurring derivatives of lipids. Naturally occurring lipids contain even numbers of carbon atoms in straight chains (from 14 to 26 atoms) with fatty acids linked by an ester or amide, either of which may be liberated on hydrolysis. An amide is simply Ammonia + an -ide, an ion picked up from an acid or free radical.

Some common Saturated Fatty Acids of animal and plant origin (adapted from W. Christie, 2002).

| Systematic name | Common name | Shorthand |
|-----------------|-------------|-----------|
| ethanoic | acetic | 2:0 |
| butanoic | butyric | 4:0 |
| hexanoic | caproic | 6:0 |
| octanoic | caprylic | 8:0 |
| decanoic | capric | 10:0 |

-The first digit before the colon indicating the number of carbon atoms in the chain and the second digit, after the colon, denoting the number of double bonds. The list goes on and on.

Note the common names in the preceding table. This is where the terms acetic and butyric come from; acetic of course being associated with acetic acid, the fatty acid in vinegar, and butyric acid, which is found in animal fats and is most pronounced in rancid butter. Caproic, caprylic, and capric acids are all derived from the Latin word *capra*, meaning goat. Caproic acid reportedly smells like wet goat, caprylic like rancid goat, and capric like fresh goat (is that possible?). These are the group of esters associated with aerobic and

anaerobic Dekkera/Brettanomyces fermentations. Horse blanket, horse sweat, foxy, feral, goaty, musky: these are all descriptors of glandular odors. This is most appropriate because all of the listed fatty acids are also products of bacterial and fungal enzymatic action on human glandular excretions. A lambic or gueuze is most accurately described in fatty acid or glandular odor terms rather than as being “estery” in odor.

While fully formed esters may be liberated as a result of enzymatic activity on various grain fractions (e.g. ethyl ester in amylopectin), they are more commonly formed from an alcohol and a grain derived fatty acid as part of a dehydration process. The alcohol and fatty acid react, often in the presence of a catalyst such as an esterase, to form the ester and a water molecule. Isoamyl acetate, the characteristic banana odor so closely associated with Weizenbiers, occurs when isoamyl alcohol reacts with acetic acid. Two other esters common to various beers are isoamyl butyrate, from the reaction of isoamyl alcohol and butyric acid which smells like pear, and ethyl acetate, made from ethyl alcohol and acetic acid, which smells like juicy fruit.

Typically, to make an ester all you need is an alcohol and a fatty acid. The number of possibilities is a function of the number of fatty acids available and the number of alcohols available. All of the even numbered C2 to C30 common saturated fatty acids are found in nature; that's 15 fatty acids. The primary alcohol in beer is ethanol. Congeners, or by-products of ethanol production, may include: Pentanol, also known as isoamyl alcohol, Butanol or butyl alcohol, and Propanol. As a group, these higher alcohols, predominantly comprised of isoamyl alcohol, are known as fusel oil or fusel alcohols. So 15 fatty acids multiplied by 4 alcohols are 60 ester possibilities. There are actually many more possibilities; this is simply for illustrative purposes. Note that fusel alcohol production increases with fermentation temperature. This is one reason why the warmer the fermentation, the greater the complexity of the ester profile.

As an aside, isoamyl alcohol in particular is affected by pitching rates. A four-fold increase in yeast results in an 80% increase in isoamyl alcohol (Van Gheluwe et al., 1975). If you are fond of strong isoamyl acetate banana, don't be shy about pitching a large volume of your favorite German Wheat Beer yeast and keeping the fermentation on the warm side.

From the catalyst side of the ester equation, the same effect seen in isoamyl acetate production should hold true for the production of just about any ester. The underlying protein responsible for this is Acetyl-Coenzyme A. Acetyl-CoA is used by yeast for growth as well as for ester production. If a small volume of yeast is pitched, Acetyl-CoA is tied up in yeast growth. If a larger volume of yeast is pitched from the start, little Acetyl-CoA is tied up in a growth phase and as a result, is free to be used by the yeast for ester production. (Cone, 2003).

As an example, the amino acid Choline alone is responsible for 36 aromatic esters, 13 of which are naturally occurring in plants. They include: anisic acid, isovanillic acid, vanillic acid, cinnamic acid, coumaric acid, and cafeic acid (Wathelet et al., 1999, [Research funded by Technologies, Research and Energy Ministry of “Region Wallonne” in Belgium, imagine that!]). The associated odors are self-explanatory. Choline is of course, yet another amino acid found in grain.

Phenol formation in weizenbier provides another fine example of an acid liberation resulting in a flavor/odor component in beer. Weizenbier production traditionally takes advantage of a 109-113°F “ferulic acid rest.” Enzymatic action in this temperature range releases ferulic acid which is bound to pentosan by an ester bond. Wheat malt has four times the ferulic acid potential of barley malt. During fermentation, ferulic acid is decarboxylated by decarboxylase to create the aromatic phenol 4-vinyl-guaiacol (4VG) which is responsible for clove aroma and flavor. Decarboxylation is nothing more than a carboxyl group (COOH) being “snipped” off the bottom of, in this case, a ferulic acid molecule. 4VG is a phenol and differs from esters in that it is not an acid/alcohol compound. Saccharomyces cerevisiae do not produce decarboxylase, therefore some wild yeast or bacteria must be present for clove phenol production to occur. (For additional information on this or just about any topic, conduct a search at Homebrew Digest: www.hbd.org). Ferulic acid incidentally is a powerful anti-oxidant which protects against ultra-violet radiation and is being studied as a beer flavor stabilizer (Walters et al., 1997).

Cellulose is the chief constituent of grain husks. In addition to the husk, all cell walls are composed of roughly 20% cellulose complexed with pentosans, beta-glucans, etc. The most pure naturally occurring cellulose is cotton fiber. Cellulose is hydrolyzed by cellulase. In brewing, because husks make up the filter

bed in the lauter and contain tannins, cellulase activity is generally not desired. There are a myriad of cellulases, mostly exogenous in origin, which seem to be most active at a pH of 5.5 and temperature of 131°F. Fortunately cellulose is tougher stuff than the gums and proteins which can be acted on by enzymes in the same temperature range.

And this deserves a note on endogenous versus exogenous enzymes. Endogenous, meaning the stuff on the inside, is there because the plant wants it to be there. It is an enzyme produced and used by the plant for some life function. Exogenous, meaning the stuff on the outside, is there because it wants to eat the plant. If a bacteria or fungus is living on a grain husk, it needs to produce an enzyme which can hydrolyze (digest) the husk food source. This bacteria or fungus then, is the source of the exogenous enzyme. If a little critter crawling around on a grain bores a hole into the starchy endosperm, it needs enzymes in its gut capable of digesting that starch. Low and behold, yet another source of exogenous amylase. And if you think this is not an important source of enzymes, stop by your friendly neighborhood field of wild oats, hand pick a bag, and look at the grain real close, preferably under magnification. Millions of little exogenous enzyme carriers will be leaping at the opportunity to hydrolyze some of your protein! Some of them and their fecal matter survive, even through the malting process, just so you can make beer.

It is thought **tannins** coexist with cellulose as an astringent barrier against bacterial and fungal attack. They are leached out of the husk by sparging too hot, sparging to too low a gravity thus raising mash pH, or made available as a result of crushing the grain too fine. Tannins complex with proline to form chill haze. Proteins can be good because they remove all kinds of bad stuff through hot and cold break and improve head retention. Tannins are bad because they are astringent and help form chill haze. If sufficient quantities of tannin and proline survive to the final product, chill haze will be visible. An excess of proline means lots of protein and possibly other good characteristics that may be associated with protein like improved head retention. Lots of protein may also include sufficient albumin and globulin to bind the tannin and remove it from solution. An excess of tannin means astringency. Better to control chill haze by controlling tannins than by going out of your way to eliminate protein sources (unless you are making a Bud clone). Ensuring proteins are properly fractionated through the use of a protein rest also helps to reduce the likelihood of chill haze.

Maltose is present in small amounts in unmalted grain. It is formed naturally in malted grain as a result of endogenous beta-amylase acting on native starch. The greater the degree of modification, the greater the amount of maltose present.

Conclusion: A lot of ground has been covered but we've only scratched the surface. Research being conducted today is helping to flesh out a better understanding of the underpinnings of long standing brewing practices. Traditional wisdom is often supported by this new research. Hopefully, one or two readers will, a month or a year from now while scrubbing out yet another corney keg, reflect on TAXI or LTP1, do a little research, and come up with an entirely new and distinct brew. To quote one of my former instructors, "You never know until you know...."

When Chad Stevens isn't busy waxing reminiscent about the effects of waxy rice amylopectin on his last batch of home malted wheat beer, he's busy flying helicopters for the U.S. Department of Homeland Security in San Diego.

Webography and Additional Reading

Note: All cites in the text are available on-line. Because some source material is not available on the web, and is not available to this author, some original works cannot be cited. Rather, subsequent works which are web available are cited. My apologies to authors of the original work.

--If wisdom were offered me with the proviso that I should keep it shut up and refrain from declaring it, I should refuse. There's no delight in owning anything unshared.

E. Philips Barker

...the mind refuses to admit that its greatest cause for pride is in its power to understand, to accept, to respect; and that modesty is the best means of enlarging its domain.

Rabindrinath Tagore

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