Small quantities of higher alcohols, principally amyl and butyl in their various isomeric forms are produced in alcoholic fermentation, and are believed to arise as degradation products of proteins in the raw materials. They are known collectively as fusel oil and in modern plants are separated in the course of rectification.

The quantity of fusel oil produced may vary from 1 to 5 gallons per 1000 gallons of industrial ethyl alcohol produced, depending on the raw material used, the conditions of fermentation, and the mode of operation of the rectifying equipment.

The following figures quoted by Kirk and Othmer are of interest:

<table>
<thead>
<tr>
<th>Source</th>
<th>Fusel Oil galls/1000 galls alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackstrap molasses</td>
<td>4 - 5</td>
</tr>
<tr>
<td>High test molasses</td>
<td>1 - 3</td>
</tr>
<tr>
<td>Corn</td>
<td>4 - 5</td>
</tr>
<tr>
<td>Degerminated corn</td>
<td>2.5 - 3.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>2 - 3</td>
</tr>
<tr>
<td>Rye</td>
<td>2 - 4</td>
</tr>
</tbody>
</table>

For the production of alcohol or rum in the West Indies, blackstrap molasses is used as the source of sugar in the raw material and a yeast organism as the fermenting agent, the species most often used being Saccharomyces cerevisiae. The blackstrap molasses consists of the residues from the evaporated cane-sugar juice, after the extraction of the crystallizable sugar and may contain from 50-60% of sugars in the form of a mixture of sucrose and invert sugar.

Kirk and Othmer declare that to start a commercial fermentation operation, a batch of molasses mixed with water, which may be several thousand gallons, must be inoculated with yeast to a concentration of 3 to 7.5 million organisms per millilitre of wash. The yeast should preferably be of pure culture, that is, of one selected strain of yeast, uncontaminated by any of the "wild" yeasts which abound in the atmosphere.
The technique of securing pure culture fermentation is to inoculate about 10 ml. of sterile wort with a pure culture of the yeast, which may be maintained on a malt-agar substrate. After incubation for about 24 hours at 25-30 °C, the culture may be used to inoculate a flask containing 200 ml. of sterile mash: after incubation this may be used to seed a sterile mash of about 4 litres capacity. Up to this point in the preparation of the starter, the work is carried out in laboratory glass containers. The next mash inoculated is of semi-plant size varying from 10-200 gallons. This fermenting mash is incorporated with the main mash.

The main mash consists of the blackstrap molasses diluted to 10-18% sugar content, and, ammonium sulphate or phosphates added to supply any deficiencies in nitrogen or phosphorus. The pH of the mash is adjusted by sulphuric acid to 4.0-5.0, the optimum pH for yeast activity, and kept at 65-75 °F. The time for complete fermentation may vary from 36 hours to 50 hours, or even longer, depending on the temperature, sugar concentration and the strain of yeast employed.

The fermented mash, or "beer" is then distilled to separate the main ethyl alcohol fraction from the higher boiling fusel oil and from other components.

There are many different forms of alcoholic distillation equipment, but each is provided with a heating unit for the fermented mash, and a rectifying column from which the various components depending on differences in boiling points may be tapped off at different levels.

The ethyl alcohol is drawn off near the top of the rectifying column, while a fusel oil fraction is taken from the lower plates of the column and is thence passed to a washer, from which a stream of the washed fusel oil is obtained.

The development of modern commercial production of industrial
alcohol of very high strength has resulted in an increase in the quantity of fusel oil rejected during rectification.

The fusel oil is contained principally in those fractions of the alcoholic distillate boiling between 105°C and 135°C: between 105°C and 120°C most of the isobutyl alcohol is obtained, whilst the amyl alcohols distil principally between 128°C and 132°C.

Forte states that if a continuous still is used for the distillation, the fusel oil tends to collect at a point in the column where the alcoholic concentration is about 43% and may be tapped off at that point; also, if batch rectification is used, the third and fourth fractions containing 15-20% of fusel oil are separately collected in a separating tank and rectifier.

Source of Higher Alcohols in Fusel Oil.

(3) Erlich has shown that the formation of higher alcohols in ordinary fusel oils arises from the action of the yeast organism upon the amino-acids which result from the decomposition of proteinaceous materials present in the mash and in the yeast itself. Leucine yielding inactive amyl alcohol, whilst isoleucine yields active amyl alcohol.

(4) However, Simmonds states that in order for the above to take place, the nitrogenous residues of the amino-acids must be split off in the form of ammonia; but this substance cannot be detected in the liquid.

To explain this Erlich concludes that the ammonia is assimilated as fast as it is formed and is utilised in the production of the nitrogenous constituents of growing yeast.

(4) Simmonds concludes also that the production of fusel oil depends upon whether the yeast derives its nutriment from amino-acids or from other nitrogen sources such as ammonium salts which yield assimilable nitrogen more readily: when the latter are present, the amino-acids are not attacked and little or no fusel oil is produced.