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## Measurement of honey quality

### Introduction

In the last 10 years honey producers, especially those in France, have seen increased competition from imported products which do not comply with the accepted description that “honey is a product harvested by bees from plants (and as such it possesses the plants characteristics); it is 100% natural and nothing should be extracted or added to it.” In some cases honey is contaminated by the addition of sugar and the search for competitively priced products sometimes drives certain importers to acquire falsified honey (as indicated by the presence of starches and ashes). Recent EC regulations (Directive 2001/110) have set new quality requirements for honey and are now being implemented in member states (for example, in the UK via Honey Regulations 2003 (SI 2003/2243).

An important factor in honey grading is colour designation. As colour is an important characteristic used by producers, packers and end-users alike, its measurement is vital in quality control processes. Indeed, it is estimated that 75% of industrial users include colour specifications in their designations; typical uses are as colouring and browning agents in various food products.

Other parameters used for specifying honey include sugar content, moisture content, water insoluble content, electrical conductivity, free acid, diastase activity and hydroxymethylfurfal (HMF) content.

### Measurement of quality and purity

#### Colour

Colour grading has been used by the honey industry for many years. In natural condition there is a continuous range of colours related to mineral content and floral source. In addition there is a connection with flavour as light coloured honey is mild whereas darker types have stronger flavours. Originally a simple optical device, the Pfund colour grader, was widely used, which compares honey with a fixed amber glass wedge and the measurements were incorporated in various standards. However, it is recognized by the industry that spectrophotometry is just as applicable and does not suffer from the instrument to instrument variability shown by Pfund graders. Thus a simple measurement of absorbance at 560nm enables a colour classification for honey to be established, as indicated in the table below



Colour names (honey)	Pfund scale mm	Mid range absorbance at 560nm
Water white	<8	0.0945
Extra white	9-17	0.189
White	18-34	0.378
Extra Light Amber	35-50	0.595
Light Amber	51-85	1.389
Amber	86-114	3.008
Dark Amber	>114	>3.1

### Diastase number

Honey consists of mainly water, glucose, fructose, sucrose, proteins and mineral salts, plus several enzymes, including diastase. This enzyme facilitates conversion of starch to maltose and is added by bees during honey production. The activity of diastase in honey is affected by storage and is sensitive to temperature increase and can thus be used as an indicator of storage time/freshness and controls during processing of the honey (HMF content is also used as an indicator of honey quality). Although natural levels are variable in honeys depending on floral source, a reduction in diastase activity from what is expected is a useful quality indicator.

Legislation has set a minimum level for diastase activity; it should not be less than 8 DN units, where 1DN unit hydrolyses 1ml of 1% starch using 1g of honey for 1 hour at 37 °C. The reference equation from the International Honey Commission gives a definition of diastase number as:

Diastase number, DN = (28.2 x absorbance change at 620nm after 10 minutes) + 2.64

### **Methods**

The honey used was obtained from a known indigenous source in the UK. The Libra S5 is visible only instrument with a wavelength range from 330 - 830 nm that may be used for simple measurements at 560nm. It also has a heated cell holder that regulates at 37 C, making it ideal for kinetics reactions such as the determination of diastase activity.

To carry out the diastase number measurement, the honey was diluted in pH 5.3 acetate buffer to make a 5% solution and then mixed with 1% starch solution. The mixture was added to a cuvette in the controlled temperature compartment of the Libra S5 which maintained the reaction of diastase with the starch substrate at 37°C. Samples were withdrawn at periods of 2.5 mins and added to iodine solution (1.3 g of iodine and 2 g of potassium iodide, dissolved in 100 ml water) which was then measured at 620 nm.

## Results

### Colour

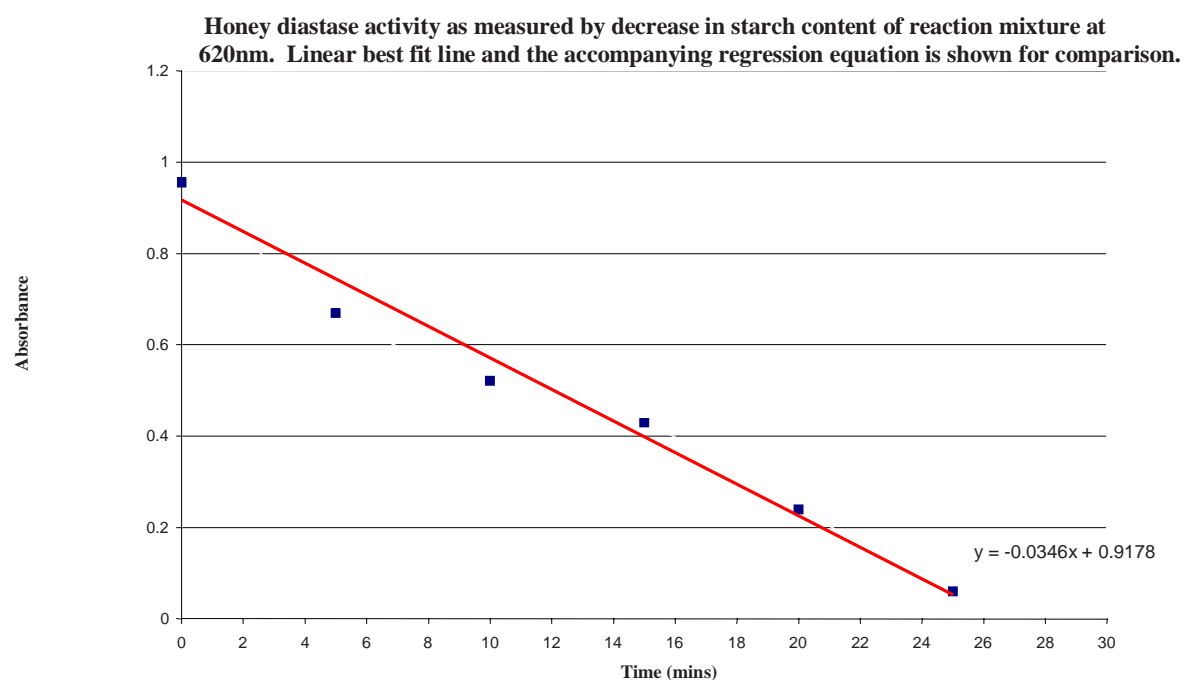
The absorbance readings for the honey solutions used were as follows:

Sample	Abs 560nm	Classification
Honey	0.576	Extra Light Amber

Note that the use of spectral measurements for colour is more precise than a single wavelength as it completely avoids dependence on a group of wavelengths which could be distorted by adulteration and omission of relevant data. Scans are readily obtained on the Libra S5. Approximations to peak area calculations after output of a complete scan to spreadsheet would enable customising the data further.

### Diastase number

Results of the standard starch solution hydrolysis with naturally occurring diastase in the honey sample are obtained on the basis that as the diastase reaction proceeds, the starch concentration decreases and this is indicated by the response from the iodine solution. The resulting absorbance of the iodine solution at 620nm with time into the reaction is shown below (results have been plotted in Excel for clarity).



For this honey sample the change in absorbance over 10 minutes from the graph is 0.37, corresponding to a DN = 13 [calculated from  $(0.37 \times 28.2) + 2.64$ ]. This is within a normal range and well above the minimum legislative requirements.

### Discussion and Conclusions

These results demonstrate the versatility of the compact instrument Libra S5 and show how it will continue the invaluable role of its predecessors for quality control in the food and drink industries. A considerable amount of data may be collected and archived from a range of samples, requiring only fairly simple preparative methods. A further advantage of the unit is the small size and convenient portable design which enables it to be used near the process, for example to check colour after filtration. Note that other Libra instruments can also be used, as they all measure in the visible range.

### Ordering Details

Libra S5 with thermostatted cell holder                      80-2115-01

### Other assays for quality control of honey

Measurements of other key parameters have been proposed by regulatory authorities. The following may also be undertaken using the Libra S5 and other instruments from the Libra range.

#### Sugar Content

Individual analyses of sugars can yield valuable information about source and floral origins. A large amount of data has been compiled by the Swiss Bee Research Centre to show the range of values for several parameters for various types of honey. For sugars this is particularly dependent on floral origin and therefore could be useful for coordination with labelling. Therefore relevant methods are:

*Glucose.* This is analysed using glucose oxidase and 4-aminophenazone, and measuring absorbance at 500nm.

*Fructose.* In this method fructose is reacted with tryptamine in HCl, at 60°C, with completion in 60 minutes. The assay is read at 518 nm and shows very low interference from other sugars.

The combined fructose and sugar content should be not less than 60g/100g for blossom honey.

#### Invertase

Like diastase, this enzyme is also susceptible to heating and storage factors so can be used also as a quality indicator. It is analysed by using the substrate p-nitrophenyl-D- glucopyranoside, with measurements at 400 nm.

### Hydroxymethylfurfural (HMF)

This can be used as an indicator of honey quality as it is a sugar breakdown product and increases with temperature and storage time. HMF has an absorbance maximum at 284nm and should be measured over the range 250-330nm; its measurement therefore requires a UV range instrument such as the Libra S12 or S22.

In general, there should be not more than 40mg/kg of HMF in honey.