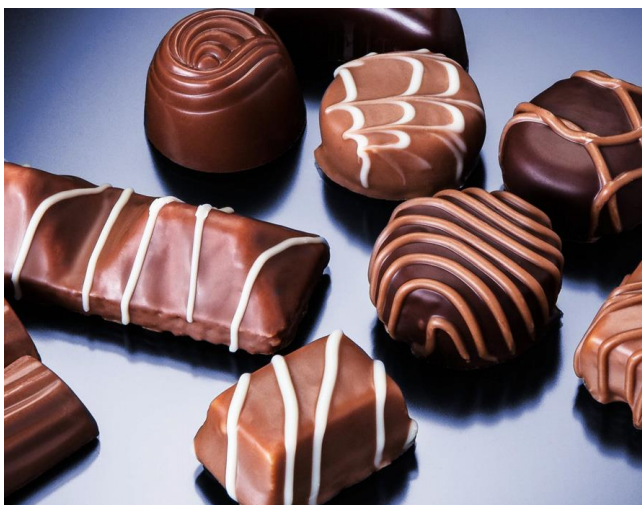


Fat Determination of Chocolate Products

Relevant for: Food

Chocolate and other cocoa products are luxury, very delicious foods. For the taste, the fat content is an important quality parameter. An ideal method for the determination of the fat content in cocoa products like chocolate, cocoa beans, or chocolate creams is refractive index measurement.



1 Everything will be fine if it is made out of chocolate

Who doesn't like chocolate? That sweet, usually brown or white confectionery, which can be liquid, paste, or solid, and is used as a flavoring ingredient in many sweets. The main components of Theobroma Cacao L. seeds are:

- **Chocolate liquor:** Ground/melted state of the nib of the cocoa bean, containing roughly equal parts cocoa butter and solids
- **Cocoa butter:** The fatty component of the bean
Cocoa solids: The remaining non-fat part of the cocoa bean, which is ground into a powder

The quality and the taste of the cocoa product strongly depends on its composition. One important quality factor is the fat content. Apart from the refractometric determination, the fat content can be determined by gravimetric analysis, which is a time-consuming method. Other alternatives are the use of high-performance liquid chromatography (HPLC) or near-infrared spectroscopy (NIRS) which are both more expensive and more time-consuming than refractometry.

2 Fat determination of cocoa products according to Leithe

The refractometric determination of the fat content of cocoa powder, cocoa beans, chocolate, and other cocoa products was published by Wolfgang Leithe and Hans Joachim Heinz in 1936.

Moreover, Leithe described refractometric analysis of the fat content in milk, seeds, and cheese. All described methods are based on the fat extraction from the samples.

The fat determination according to Leithe is quick and easy. Depending on the sample, it takes between 10 min and 20 min. The fat content in cocoa beans, cocoa mass, and cocoa products is indirectly determined via refractive index measurement after fat extraction in 1-bromonaphthalene.

Further extraction solvents have been tested during the last century. Another method describes the use of tricresylphosphate at 100 °C (Nadj and Weeden, 1966). In 1995, the fat content of pre-fried French fries was determined according to Leithe by using 1-chloronaphthalene (Eschert and Baumann, 1995).

An extraction solvent is ideal if it has good solvent properties and low volatility and toxicity. Moreover, the refractive index of the solvent should be as different as possible from the refractive index of the fat being extracted (Nadj and Weeden, 1966).

3 Measuring principle

After fat extraction the fat content in 1-bromonaphthalene is determined. This is used to calculate the fat content of the sample. The change in the refractive index between the 1-bromonaphthalene and 1-bromonaphthalene-fat mixture correlates with the percentage of fat of the sample.

As a prerequisite the density and refractive index of the pure fat ($\rho_{\text{cocoa butter}} = 0.913 \text{ g/cm}^3$, $RI_{\text{cocoa butter}} = 1.4647$) should be known or measured in advance.

The sample preparation requires 2.0 g of the chocolate sample which is crushed and well mixed with 4.45 g of 1-bromonaphthalene. The refractive index of the 1-bromonaphthalene as well as of the mixture has to be determined. A higher fat content of the sample results in a higher difference of the refractive index between 1-bromo-naphthalene and the mixture. Together with the density value and the refractive index of the pure fat the final concentration of fat in the sample can be determined using the refractometer.

4 Measuring with Abbemat refractometers from Anton Paar

The Abbemat refractometers from Anton Paar (Fig. 1) are ideal for measuring the refractive index with high accuracy. Therefore, they can be used for the fat content determination in cocoa products.



Figure 1: The Abbemat refractometer family from Anton Paar.

If using an Abbemat refractometer from the Performance Plus line (Abbemat 350, 550) or Heavy Duty line (Abbemat 450, 650), the percentage of fat will be directly displayed on the Abbemat screen (Fig. 2).

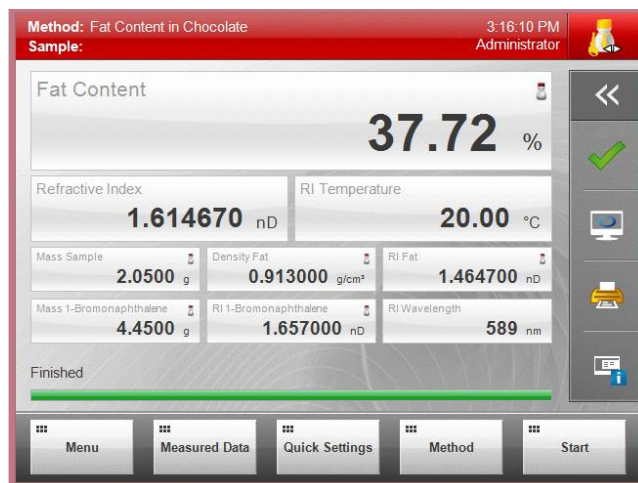


Figure 2: After pressing "Start", all relevant parameters have to be entered. After pressing "Start" again the fat content (%) is immediately calculated and directly shown on the screen of the Abbemat 350, 450, 550 or 650 refractometer from Anton Paar.

Please note, that the corresponding method "Fat Content in Chocolate" is not implemented as a standard method on the refractometers. Ask your Anton Paar representative to send you the corresponding user function which can be easily implemented on your refractometer.

5 Measurement procedure

The refractometer method "Fat Content in Chocolate" is only available for the Abbemat Performance Plus line and the Heavy Duty line.

5.1 Samples

Cocoa beans, cocoa mass, cocoa powder, chocolate, chocolate creams

5.2 Safety precautions

This method does not contain any safety instructions. It is the responsibility of the user of this method to establish appropriate health and safety practices and to determine the applicability of regulatory limitations prior to its use.

5.3 Instrument preparation

The measuring prism of the Abbemat must be kept clean. Clean the prism of the refractometer with distilled water and acetone. The instrument should be calibrated using a refractive index standard provided by Anton Paar. The temperature control of the liquid being examined and the cleanliness of the prism

should be frequently checked by determining the refractive index of distilled water, which is 1.332986 n_D at 20 °C and 589.3 nm wavelength.

5.4 Sample preparation

Cocoa bean:

- Weigh out 2.0000 g of the sample as accurately as possible (± 0.05 g) and note the value
- Measure the refractive index of the pure extracting agent 1-bromonaphthalene at 20 °C and 589.3 nm, which should be around 1.657
- Measure the refractive index at 20 °C and 589.3 nm, which should be around 1.465, and the density at 20 °C of the pure cocoa fat (density value can be taken from the literature, cocoa fat = 0.913 g/cm³)
- In order to get a good extraction, crush the sample as good as possible, then add 4 g sea sand and 4.45 g (± 0.05 g) of 1-bromonaphthalene
- Mix everything thoroughly (vortex mixer) until the mixture is homogeneous
- Filter by using dry filter paper or accelerate the filtering process by using a centrifuge

Cocoa mass, cocoa powder, chocolate

- Weigh out 2.0000 g of the sample as accurately as possible (± 0.05 g) and note the value
- Measure the refractive index of the pure extracting agent 1-bromonaphthalene at 20 °C and 589.3 nm, which should be around 1.657
- Measure the refractive index at 20 °C and 589.3 nm and the density of the pure fat (density can be taken from the literature as well, cocoa fat = 0.913 g/cm³)
- In order to get a good extraction, crush the sample as good as possible, then add 4.45 g (± 0.05 g) of 1-bromonaphthalene
- Mix everything thoroughly (vortex mixer) for 2 min until the mixture is homogeneous; use a mortar if necessary
- Filter by using dry filter paper or accelerate the filtering process by using a centrifuge

Table 1: Density of different fats and oils at 20 °C in g/cm³ (Handbook of Food Science, Technology and Engineering 2005).

Fat	Density at 20 °C and 1 atm in g/cm ³
Cocoa butter	0.913
Milk / butter fat	0.911
Palm kernel oil	0.9252
Coconut oil	0.924
Hazelnut oil	0.912 - 0.917

After sample preparation the filtered extract is applied to the measuring prism of the Abbemat. After pressing "Start" the predefined values are entered and after pressing "Start" again the refractive index of the filtrate is measured. The fat content (%) is directly shown on the screen of the Abbemat refractometer (Fig. 2).



Figure 3: Milk chocolate homogenized in 1-bromonaphthalene (left) and after filtration (right).

5.5 Calculation of the fat content

As shown in Figure 2, the fat content can be directly displayed on the Abbemat 350, 450, 550 and 650 refractometer screen.

In case of using an Abbemat 200, 300 or 500 the fat content can be manually calculated after measuring the refractive index of all relevant substances. The following formula can be used:

Fat Content [%] =

$$\frac{\rho_F * m_B}{m_S * \rho_B} * \frac{n_B - n_{BF}}{n_{BF} - n_F} * 100$$

- ρ_F = density of fat (0.913 g/cm³ for cocoa butter)
- ρ_B = density of 1-bromonaphthalene (1.483 g/cm³)
- m_B = mass of weighed 1-bromonaphthalene
- m_S = mass of weighted sample
- n_B = the refractive index of 1-bromonaphthalene at 20 °C and 589.3 nm (about 1.657)
- n_F = the refractive index of the fat at 20 °C and 589.3 nm (1.465 for cocoa butter, 1.470 for hazelnut fat)
- n_{BF} = the refractive index of the filtered extract at 20 °C and 589.3 nm

6 Appendix: What's behind the formula?

The refractive index n of two mixed liquids with their refractive indices n_1 and n_2 and their volumes V_1 and V_2 which do not interact with each other can be described by the following formula:

$$V_2 = V_1 * \frac{n_1 - n}{n - n_2}$$

The density ρ and the mass m have the following relation with the volume:

$$\rho_2 * V_2 = m_2$$

Therefore, the density ρ_2 is added on both sides of the formula:

$$m_2 = V_2 * \rho_2 = \rho_2 * V_1 * \frac{n_1 - n}{n - n_2}$$

As the percentage of fat, which can also be referred to as mass of fat per mass of sample multiplied by hundred, should be calculated, both sides still need to be divided by the mass of the sample.

Fat content [%] =

$$100 * \frac{m_2}{m_S} = 100 * \frac{\rho_F * V_B}{m_S} * \frac{n_B - n_{BF}}{n_{BF} - n_F}$$

7 References

Baumann, B., Eschert, F. (1995). Mass and heat transfer during deep-fat frying of potato slices- 1. Rate of drying and oil uptake. *LWT- Food Science and Technology* 28(4):395-403.

Hui Y. A. (2005). *Handbook of Food Science, Technology and Engineering*. Vol. 4, CRC Press, p. 9-13.

Leithe, W. (1936). Refraktometrische Fettbestimmung in Milch und Milcherzeugnissen. *Zeitschrift für Untersuchung der Lebensmittel*, Vol 58, p. 245 - 251.

Leithe, W., Heinz, H.J., (1936). Refraktometrische Fettbestimmung in Kakaowaren. *Zeitschrift für Untersuchung der Lebensmittel*, No. 72, p. 414-418.

Nadj, L., Weeden, D. (1966). Refractometric estimation of total fat in chocolate type products. *Analytical chemistry*, Vol 38, No1, p.125-126.

Rochel, F., Braselmann, H. (1961), Untersuchungen über die refraktometrische Milchfettbestimmung nach Leithe. *Die Nahrung*, Heft 4, p. 411-418.

Stanley, W. A. et al., (1937), Refractometric determination of fat in chocolate. *Industrial and engineering chemistry*, Vol. 9, No. 3, p. 132-135.

Contact Anton Paar GmbH

Tel: +49 511 40095-0 | www.anton-paar.com
application-optotec@anton-paar.com