Univerza v Mariboru

FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

Laboratory for Separation Processes and Product Design

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MODERN TECHNOLOGIES FOR FOOD PROCESSING

Instructions for laboratory work

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1. MICROENCAPSULATION OF BIOLOGICALLY ACTIVE COMPOUNDS

1.1. MICROENCAPSULATION WITH NATRIUM ALGINATE

1.1.1. Theoretical background

Microencapsulation is a process by which a core, i.e. bioactive or functional ingredient, is packaged within a secondary material to form a microcapsule. The secondary material, known as the encapsulant, matrix or shell, forms a protective coating or matrix around the core, isolating it from its surrounding environment until its release is triggered by changes in its environment (Sanguansri et al., 2010). Microcapsules are small vesicles or particulates that may range from sub-micron to several millimeters in size (Fang et al., 2010). Many morphologies can be produced for encapsulation; typical shapes of microcapsules are presented in **Figure 1.1**.

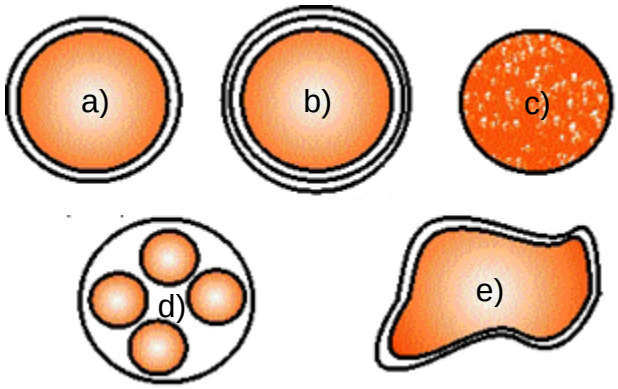

Figure 1.1: Examples of microcapsule shapes: a) simple mononuclear spherical b) mononuclear multilayer, c) aggregate – many cores embedded in a matrix, d) spherical multicore, e) irregular (MicroInno, 2011).

The primary reasons for microencapsulation of food ingredients are to (Sanguansri et al., 2010):

1) protect the core material from adverse environmental conditions such as undesirable effects of oxygen, moisture and light that can cause their degradation during processing and storage,
2) avoid undesirable interactions of the bioactive compounds with other food components or chemical reactions that can lead to degradation of the bioactive, with possible undesirable consequences on taste and odour as well as negative health effects,
3) control release characteristics of the core, including its delivery to the desired site after ingestion.

Many microencapsulation methods have been developed and many substances may be used to coat or encapsulate solids, liquids or gases of different types and properties. The choice of method used for microencapsulation depends on the properties of the core, the encapsulant materials and the requirements of the target food application. One possible encapsulation technique is microencapsulation of the core in an aqueous solution of sodium alginate.

1.1.2. Basic principle

Alginate is an anionic linear polysaccharide with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. It is widely distributed in the cell walls of brown algae. The alginate is highly hygroscopic and, when in contact with water, forms a viscous medium with properties similar to a gel. The specificity of sodium alginate is its extremely fast cross-linking reaction with the addition of calcium ions (**Figure 1.2**) (Samanta et al., 2014).

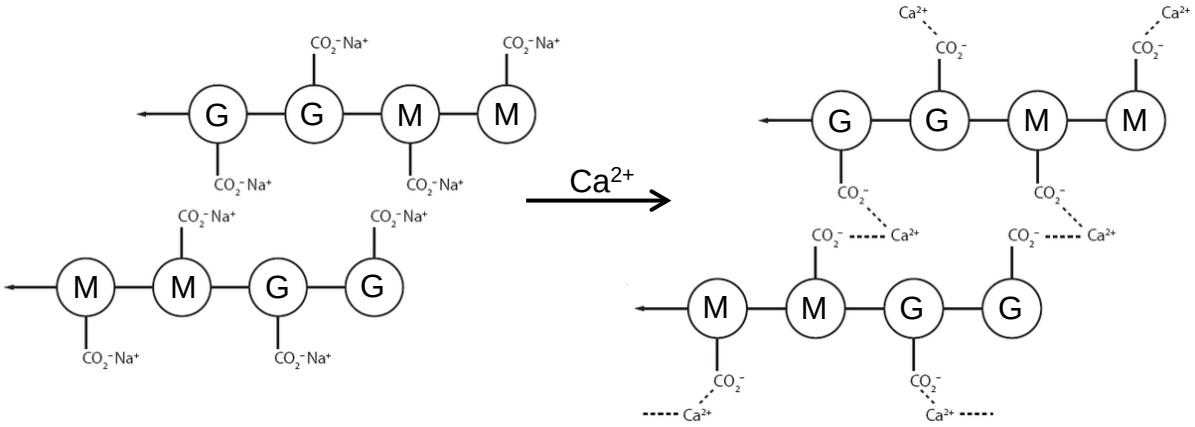

Figure 1.2: Alginate polymerization by addition of calcium ions.

Upon direct contact of the sodium alginate solution with an aqueous medium containing calcium ions, a membrane is formed that encapsulates the core and protects it against external influences, thereby increasing the stability of the food.

Bioactive compound: curcuma extract

Turmeric (*Curcuma longa*) is a plant of tropical origin of the ginger family. The rhizome of turmeric is an important source of a yellow natural pigment, which in the past has been used as a spice, a colouring agent in the food industry, for household medicine usage and as an insect repellent. Turmeric is one of the most popular medicinal herbs, with a wide range of pharmacological activities, such as antioxidant, antiprotozoal, antivenom, antimicrobial, anti-inflammatory, antiproliferative, antiangiogenic, antitumor and antiageing (Perko et al., 2015).

The yellow colour, which is characteristic for turmeric rhizome, is due to the presence of 3-5% of curcuminoids. Curcuminoids, represented by curcumin (50-60%), demethoxycurcumin (20-30%) and bisdemethoxycurcumin (7-20%) [Jayaprakasha et al., 2006; Chatterjee et al., 1999] (**Figure 1.3**) have poor stability and low aqueous solubility [Martins et.al, 2013]. With appropriate protection of these components, their stability and the shelf life of the product can be extended.

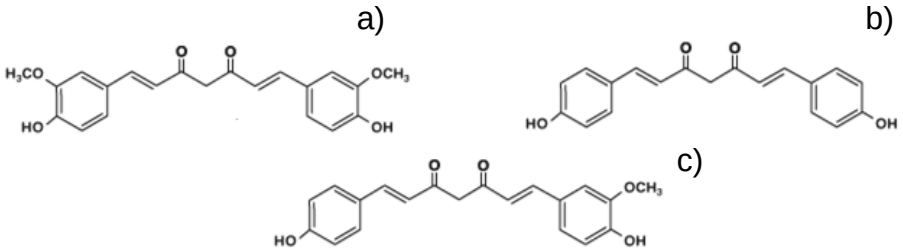

Figure 1.3: Molecular structure of curcumin (a), demethoxycurcumin (b) and bisdemethoxycurcumin (c).

1.1.3. Materials and reagents

- 10% aqueous solution of curcuma extract,
- sodium alginate,
- $CaCl_2$.

1.1.4. Accessories

- 100 mL and 1000 mL beakers,
- measuring cylinder (100 mL),
- 10 mL containers,
- 20 mL syringe with a steel cannula,
- sieve,
- spatula, tweezers, spoon.

1.1.5. Apparatus

- analytical balance,
- magnetic stirrer.

1.1.6. Experimental procedure

The beaker is filled with 25 mL of deionized (Milli-Q) water. The beaker is placed on the magnetic stirrer. The mixing rate should be 550 rpm. 0.25 g of sodium alginate are weighed and slowly added into the beaker and mixed for 30 min. In the meantime, 500 mL of 0.5% $CaCl_2$ solution is prepared and filled in a 1000 mL beaker.
After stirring the alginate solution, approximately 6.5 g of curcumin extract is added to the beaker and stirred again for at least 10 min to completely homogenize the mixture. The aqueous solution of curcumin extract and sodium alginate is filled in a syringe with a steel cannula and dripped slowly into the calcium chloride bath (**Figure 1.4**). Upon contact of the drop with the calcium media, a crosslinking occurs resulting in the formation of spherical capsules. After the formation of the last capsule, wait 2 minutes. The capsules are then separated by sieve and rinsed with distilled water. Capsules should be separated in such a way that they do not touch!

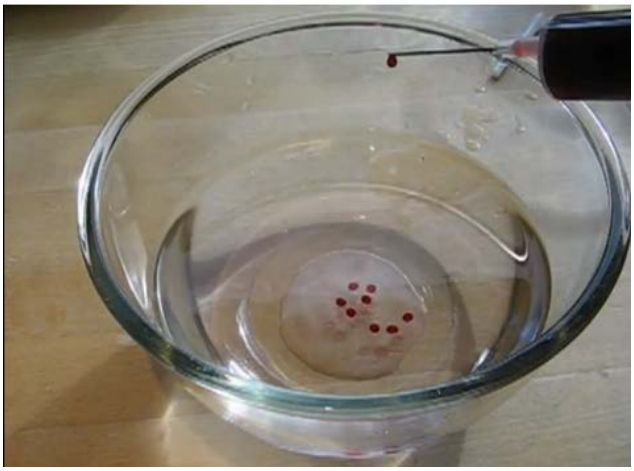

Figure 1.4: Preparation of microcapsules by dripping the solution of active compound and alginate into the $CaCl_2$ solution (Lersch, 2007).

1.2. FREEZE DRYING

1.2.1. Basic principle

Freeze-drying or lyophilisation is a dehydration process typically used to preserve a biological or organic material that is thermolabile and could be degraded when heated. In this way, the structure and composition of the material are preserved. Freeze-drying is based on freezing the material and reducing the surrounding pressure. After that a sufficient amount of heat is supplied to allow the frozen water in the material to sublime directly from the solid phase to the gas phase. Lyophilization is a slow drying process and, compared to conventional thermal drying processes, up to 3 times more energy is consumed.

1.2.2. *Accessories*

- Petri dish,
- parafilm,
- spatula.

1.2.3. *Apparatus*

- Liophilizator LIO 2000 PNS (**Figure 1.5**).

Figure 1.5: Liophilizator LIO 2000 PNS (Kambič, 2005).

1.2.4. *Experimental procedure*

Microcapsules on the sieve are placed in the lyophilization chamber and the chamber is closed. The main switch is turned on. On the working window a drying program is selected with the drying temperature set to -20°C. The final pressure in the lyophilization chamber should be lower than 0.1 kPa. At these adjusted working conditions, the sublimation of water from microcapsules will start. Depending on the amount of material, drying can last from a few hours to a few days.
After the drying, the capsules are scraped off the sieve and kept in a refrigerator in Petri dish for a week.

1.3. ANALYSIS OF QUALITY OF ENCAPSULATED PRODUCT: ABILITY OF INHIBITION OF FREE RADICALS

1.3.1. Basic principle

The antioxidative activity of the extract is determined using a stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The methanolic solution of the DPPH radical is violet coloured and has an absorption maximum at a wavelength of 515 nm. By reaction of DPPH with an antioxidant $DPPH_2$ is produced which does not absorb at wavelength of 515 nm, what can be seen in the change in colour. Changes in the colour of the DPPH radical solution (from strongly violet to light yellow) are measured by a spectrophotometer at a wavelength of 515 nm. To determine the antioxidant activity, a methanolic DPPH radical solution is used.

1.3.2. Chemicals and reagents

- 2,2-diphenyl-1- picrylhydrazyl (DPPH),
- methanol,
- 6×10^{-5} M solution of DPPH in methanol.

1.3.3. Accessories

- flasks (100 and 10 mL),
- pipettes,
- dark containers (10 mL),
- spatula.

1.3.4. Apparatus

- analytical balance,
- ultrasonic bath,
- spectrophotometer.

1.3.5. Experimental procedure

The analytical method is described in the literature (Majhenič et al., 2007). 10 mg of the sample is weighed into a 10 mL flask and diluted to the mark with methanol. The solution is mixed and/or placed on the ultrasonic bath to dissolve the extract.
The solution of DPPH in methanol (6×10^{-5} M) is prepared daily, before UV measurements. 3 mL of this solution are mixed with 77 µl extract solution in the flask. The sample is kept in the dark for 15 min at room temperature and then absorbance is measured at 515 nm (changes in colour, from violet to light yellow) using an UV-Vis spectrophotometer against a blank. The blank is prepared using the same procedure as described for the extract solution

except that 77 µL of distilled water is used instead and is measured immediately at 515 nm. The antioxidant activity is expressed as the percentage of inhibition of DPPH radicals towards the blank and is calculated using eq. 1.1:

$$\%\ \text{inhibition} = \frac{A_c^0 - A_s^{15}}{A_c^0} \qquad (1.1)$$

where A_c^0 represents the absorbance of the blank measured immediately after the addition of the DPPH solution and A_s^{15} the absorbance of the sample after a 15 min incubation in the dark.

1.4. Questions and tasks:

- How could the size of microcapsules be increased?
- Why is the freeze-drying procedure in the case of microcapsules more appropriate than dry-air thermal drying?
- Which drying process would be also suitable for drying the microcapsules?
- At what concentration of curcumin extract is the % inhibition of DPPH radicals 50% (IC_{50})?
- What is the % inhibition of the prepared extract solution after one week of storage in the refrigerator?
- What is IC_{50} of encapsulated curcumin extract after one week?

2. THERMAL PROPERTIES OF FOODS

Thermal properties of foods can be determined by using empirical equations if the food composition is known. The content of carbohydrates, proteins, fats, water and ash in the food can be determined by standard analytical methods that are described in the following subchapters.

2.1. Analysis of water content

2.1.1. Apparatus

– thermo-balance Mettler-Toledo HB43-S, Switzerland (**Figure 2.1**).

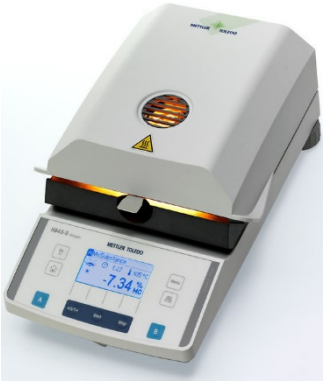

Figure 2.1: Thermobalance Mettler Toledo HB43-S for analysis of water content in the material.

2.1.2. Basic principle

The Mettler Toledo HB43-S is a halogen moisture analyzer that works on the thermogravimetric principle. At the start of the measurement, the moisture analyzer determines the weight of the sample, the sample is then quickly heated by the integral halogen heating module and the moisture vaporizes. During the drying process, the instrument continually measures the weight of the sample and displays the reduction in moisture. Once drying has been completed, the moisture or solids content of your sample is displayed as the final result.

2.1.3. *Experimental procedure*

Place the empty aluminium sample pan in the sample pan handler and weigh approximately 4 g of sample into the sample pan. Dry the sample at 115°C. When drying is complete the moisture content of your sample is displayed.

2.2. Analysis of ash content

2.2.1. *Basic principle*

– Dry ashing of the sample at a temperature of 550°C.

2.2.2. *Theoretical background*

In the dry ashing procedure, the sample is heated and maintained for a specific time at high temperature (> 500°C) in the presence of air so that complete combustion and conversion of sample into ash is achieved. In the presence of the oxygen in air, water and other volatile materials are vaporized and organic substances are burned to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. The ash content is a measure of the total amount of minerals present within a food.

2.2.3. *Accessories*

– crucible,
– muffled furnace,
– Bunsen burner,
– desiccator.

2.2.4. *Experimental procedure*

Approximately 3 g of the sample are placed into a pre-heated, cooled and weighed crucible. The crucible is firstly carefully heated over the flame of a Bunsen burner and afterwards in the furnace at a temperature of 550°C until the ash is light gray. Then the material is cooled in a desiccator and quickly weighed (Plestenjak et al., 1996). The ash content in the sample is calculated using Equation 2.1.

$$w(\text{ash}) = \frac{m(\text{ash})}{m(\text{sample})} \tag{2.1}$$

where: m(ash) – mass of ash [g]

m(sample) – mass of sample [g]
w – mass fraction

2.3. Analysis of fat content

2.3.1. Basic principle

– Extraction by Soxhlet apparatus

2.3.2. Theoretical background

The Soxhlet apparatus is a laboratory apparatus for the extraction of components from solid material with a volatile organic solvent. The apparatus consists of a distillation flask, reflux condenser and cylindrical part - percolator with a siphon mechanism (Knez et al., 2000).
The solvent evaporates from the flask and the vapours are condensed in the condensator. The condensate drops onto the thimble with material in the cylindrical part and dissolves the solutes. When the solution fills the chamber in cylindrical part the chamber is emptied by the siphon and the extract solution is collected in the distillation flask. This cycle may be repeated until the desired compounds are separated from the solid material and are concentrated in the distillation flask. The advantage of this extraction method is that the solid material comes into contact with many portions of fresh solvent that is recycled. Furthermore, the temperature in the cylindrical part of the device is lower than in the distillation flask, which is favorable for the extraction of thermally sensitive substances. The process is more economical, since it is not necessary to separate the extract solution from the residual solid material (Knez et al., 2000).

2.3.3. Reagents and accessories

– petroleum ether,
– Soxhlet apparatus,
– 250 mL round bottom flask,
– 250 mL measuring cylinder,
– filter paper,
– water bath,
– thermometer,
– rotary evaporator,
– desiccator.

2.3.4. Experimental procedure

Approximately 10 g of ground sample are placed into a thimble made of filter paper and inserted into a Soxhlet apparatus. 250 mL of petroleum ether are filled in a clean and pre-weighed flask, boiling stones are added and the flask is connected to the apparatus. The solvent in a flask is heated by a water bath and extraction is performed at a constant temperature (normal boiling point, approx. 60°C) until the solvent is colourless. After the extraction is completed, the solvent from the extraction mixture is evaporated by the rotary evaporator and the mass of the extract obtained is determined gravimetrically. The content of the fats in the sample is calculated by the equation 2.2.

$$w(\text{fats}) = \frac{m(\text{extract})}{m(\text{sample})} \tag{2.2}$$

where: m(extract) – mass of extract [g]
m(sample) – mass of sample extracted [g]
w – weight fraction

2.4. Analysis of total carbohydrates content

2.4.1. Basic principle

– phenol-sulfuric acid colorimetric method (DuBois et al., 1956).

2.4.2. Theoretical background

The Phenol–Sulfuric Acid method of DuBois et al. (1956) is a reliable and widely used colorimetric method for carbohydrate analysis. The method is based on dehydration of hydrolyzed saccharides to furfural derivatives during reaction with concentrated sulfuric acid. Further reaction of the furfural derivatives with phenol forms coloured complexes that absorb light in the visible range, with a maximum absorbance at wave-length of 490 nm (Albalasmeh et al., 2013).

2.4.3. Reagents and accessories

– conc. H_2SO_4,
– 5% aqueous solution of phenol,
– glucose (99.5%),
– Milli-Q water,
– flask,
– measuring cylinder,

- pipette,
- UV-Vis spectrophotometer.

2.4.4. Experimental procedure

Calibration curve:

Standard glucose solution with a concentration of 10 mg/mL is prepared and diluted as shown below:

	pure Milli-Q water	→	γ_1 = 0.00 mg/mL
50 µL glucose (10 mg/mL) +	9950 µL Milli-Q water	→	γ_2 = 0.05 mg/mL
100 µL glucose (10 mg/mL) +	9900 µL Milli-Q water	→	γ_3 = 0.10 mg/mL
150 µL glucose (10 mg/mL) +	9850 µL Milli-Q water	→	γ_4 = 0.15 mg/mL
200 µL glucose (10 mg/mL) +	9800 µL Milli-Q water	→	γ_5 = 0.20 mg/mL
250 µL glucose (10 mg/mL) +	9750 µL Milli-Q water	→	γ_6 = 0.25 mg/mL

Standard reagents are added to the prepared glucose solutions. To a 0.4 mL aqueous solution of carbohydrate, first 0.2 mL of aqueous solution of 5% phenol and immediately after that 1 mL of concentrated H_2SO_4 are added. The solutions are allowed to stand for 10 min at room temperature. Then, they are stirred for 30 s with the vortex and again allowed to stand for 20 minutes in a water bath at room temperature. An intensive orange colour is developed. The absorbances of the solutions are measured by UV-Vis spectrophotometer at 490 nm and from the calibration curve ($A = f(\gamma_i)$) the equation of the calibration line ($A = k\gamma + n$) is determined.

Sample analysis:

Preparation of the sample for analysis:

1. Before analysis, fats have to be removed from the material, since they impede the determination of total carbohydrates. In order to remove fats from the sample, the Soxhlet method is used (section 2.3).
2. In order to facilitate the preparation of aqueous carbohydrate solution, the defatted material is sieved and a fraction of particles with a diameter of ≤ 0.5 mm is selected for further work.

Approximately 4 mg of the sample are weighed and suspended in 8 mL of Milli-Q water. Then, 4 mL of a 5% aqueous solution of phenol and immediately thereafter 20 mL of concentrated H_2SO_4 are added. After that the same procedure is used as described above for the calibration curve.

The content of carbohydrates (CH) in the sample is calculated using the calibration curve. The following equations are used:

$$\gamma(\text{sample solution}) = \frac{A - n}{k} \tag{2.3}$$

$$w(\text{CH}) = \frac{\gamma(\text{sample solution}) \cdot V(\text{H}_2\text{O})}{m_{\text{sample}}} \tag{2.4}$$

where: γ – concentration of CH [mg/mL]
A – absorbance
k – slope of the calibration line
n – intercept of the calibration line
m_{sample} – mass of sample weighed for analysis [mg]
V (H_2O) – volume of water [mL] used for preparation of sample solution

2.5. Analysis of proteins content

2.5.1. Basic principle

– Bradford colorimetric method (Bradford, 1976)

2.5.2. Theoretical background

The Bradford colorimetric method for determination of proteins involves the binding of Coomassie Brilliant Blue G-250 to protein. The binding of the dye to protein causes a shift in the absorption maximum of the dye from 465 to 595 nm, and it is the increase in absorption at 595 nm which is monitored.

2.5.3. Reagents and accessories

– Reagent: The assay reagent is made by dissolving 100 mg of Coomassie Blue G250 in 50 mL of 95% ethanol. The solution is then mixed with 100 mL of 85% phosphoric acid and made up to 1 L with distilled water.
– protein standard: bovine serum albumin (BSA),
– spectrophotometer and cuvettes,
– pipettes.

2.5.4. Experimental procedure

Calibration curve:

A standard albumin solution with a concentration of 10 mg/mL is prepared and diluted as shown below:

	pure Milli-Q water	→	γ_1 = 0.00 mg/mL
20 μL albumin solution (10 mg/mL) +	980 μL Milli-Q water	→	γ_2 = 0.20 mg/mL
40 μL albumin solution (10 mg/mL) +	960 μL Milli-Q water	→	γ_3 = 0.40 mg/mL
60 μL albumin solution (10 mg/mL) +	940 μL Milli-Q water	→	γ_4 = 0.60 mg/mL
80 μL albumin solution (10 mg/mL) +	920 μL Milli-Q water	→	γ_5 = 0.80 mg/mL
100 μL albumin solution (10 mg/mL) +	900 μL Milli-Q water	→	γ_6 = 1.00 mg/mL
120 μL albumin solution (10 mg/mL) +	880 μL Milli-Q water	→	γ_6 = 1.20 mg/mL

To 20 μL of prepared albumin solution, 1 mL of Bradford reagent is added and mixed in a test tube using a vortex for 30 s. After shaking, the absorbance of the solution is measured at a wavelength of 595 nm using an UV-Vis spectrophotometer (Cary 50, Varian, USA) against a blank. The blank is prepared using the same procedure as described for the albumin solution except that distilled water is used instead of solution. From the calibration curve ($A = f(\gamma_i)$) the equation of the calibration line ($A = k\gamma + n$) is determined.

Sample analysis:

Preparation of the sample for analysis:

For the analysis of total protein content, the sample is prepared in a similar way as in the case of carbohydrate analysis. For the analysis, defatted material with particle diameter of ≤ 0.5 mm is used.

Approximately 4 mg of the sample are weighed and suspended in 8 mL of Milli-Q water. To 1 mL of prepared extract solution, 1 mL of Bradford reagent is added and mixed in a test tube using a vortex for 30 s. After shaking, the absorbance of the solution is measured at a wavelength of 595 nm using an UV-Vis spectrophotometer against a blank.

The quantification of total protein content in an extract solution is performed using a calibration curve obtained with albumin bovine serum (BSA) (use eq. 2.3), and the total protein content in the sample is calculated using eq. 2.5:

$$w(\text{protein}) = \frac{\gamma(\text{sample solution}) \cdot V(\text{H}_2\text{O})}{m_{\text{sample}}} \tag{2.5}$$

2.6. Calculations

2.6.1. Calculation of energy value (EV) of sample and energy fractions of the energy-yielding compounds (EF)

Calculation is based on the assumption that the energy values of the energy-yielding compounds are 17 kJ/g (4.0 kcal/g) for proteins, 37 kJ/g (9.0 kcal/g) for fats and 17 kJ/g (4.0 kcal/g) for carbohydrates, regardless of the food in which they are found. Energy value (*EV*) of the sample and energy fractions (*EF*) of individual compounds are then calculated using the following equations (Plestenjak et al., 1996):

$$EV(\text{proteins})[\text{kJ}/100\text{ g sample}] = 100 \cdot w(\text{proteins}) \cdot 17 \quad (2.6)$$

$$EV(\text{fats})[\text{kJ}/100\text{ g sample}] = 100 \cdot w(\text{fats}) \cdot 37 \quad (2.7)$$

$$EV(\text{carbohydrates})[\text{kJ}/100\text{ g sample}] = 100 \cdot w(\text{carbohydrates}) \cdot 17 \quad (2.8)$$

$$EV(\text{sample})[\text{kJ}/100\text{ g sample}] = EV(\text{proteins}) + EV(\text{fats}) + EV(\text{carbohydrates}) \quad (2.9)$$

Energy fractions (*EF*) of individual compound *i* (proteins, fats and carbohydrates) are calculated by the following equation:

$$EF(i)[\%] = 100\frac{EV(i)}{EV(\text{sample})} \quad (2.10)$$

2.6.2. Calculations of thermal properties

Equations that allow calculation of the thermal properties of food are (Ibarz et al., 2003):

$$\lambda = \sum_i \left(\lambda_i X_i\right) \quad (2.11)$$

$$c_p = \sum_i \left(c_{p_i} w_i\right) \quad (2.12)$$

$$\rho = \frac{1}{\sum_i \left(\frac{w_i}{\rho_i}\right)} \quad (2.13)$$

$$\alpha = \sum_i \left(\alpha_i X_i\right) \quad (2.14)$$

$$\alpha = \frac{\lambda}{\rho\, c_p} \quad (2.15)$$

where: λ_i – thermal conductivity of component *i* [W/m°C]
c_{pi} – specific heat of component *i* [J/kg°C]
α – thermal diffusivity [m^2/s]
w_i – mass fraction of component *i*
ρ_i – density of component *i* [kg/m^3]
X_i – volumetric fraction of component *i*
T – temperature [K]

The volumetric fraction of the component *i* is given by the expression:

$$X_i = \frac{w_i / \rho_i}{\sum_i w_i / \rho_i} \tag{2.16}$$

Equations for calculating thermal properties of main components of foods are summarized in **Tables 2.1** and **2.2.**

Table 2.1: Equations for calculating thermal properties of food components (Ibarz et al., 2003).

Thermal Property	Component	Equation as a Function of Temperature
λ (W/m·°C)	Carbohydrate	$\lambda = 0.20141 + 1.3874 \times 10^{-3} T - 4.3312 \times 10^{-6} T^2$
	Ash	$\lambda = 0.32962 + 1.4011 \times 10^{-3} T - 2.9069 \times 10^{-6} T^2$
	Fiber	$\lambda = 0.18331 + 1.2497 \times 10^{-3} T - 3.1683 \times 10^{-6} T^2$
	Fat	$\lambda = 0.18071 + 2.7604 \times 10^{-3} T - 1.7749 \times 10^{-7} T^2$
	Protein	$\lambda = 0.17881 + 1.1958 \times 10^{-3} T - 2.7178 \times 10^{-6} T^2$
$\alpha \cdot 10^6$ (m^2/s)	Carbohydrate	$\alpha = 8.0842 \times 10^{-2} + 5.3052 \times 10^{-4} T - 2.3218 \times 10^{-6} T^2$
	Ash	$\alpha = 1.2461 \times 10^{-1} + 3.7321 \times 10^{-4} T - 1.2244 \times 10^{-6} T^2$
	Fiber	$\alpha = 7.3976 \times 10^{-2} + 5.1902 \times 10^{-4} T - 2.2202 \times 10^{-6} T^2$
	Fat	$\alpha = 9.8777 \times 10^{-2} + 1.2569 \times 10^{-4} T - 3.8286 \times 10^{-8} T^2$
	Protein	$\alpha = 6.8714 \times 10^{-2} + 4.7578 \times 10^{-4} T - 1.4646 \times 10^{-6} T^2$
ρ (kg/m^3)	Carbohydrate	$\rho = 1.5991 \times 10^3 - 0.31046 T$
	Ash	$\rho = 2.4238 \times 10^3 - 0.28063 T$
	Fiber	$\rho = 1.3115 \times 10^3 - 0.36589 T$
	Fat	$\rho = 9.2559 \times 10^2 - 0.41757 T$
	Protein	$\rho = 1.3299 \times 10^3 - 0.51840 T$
$\hat{C}_p$ (kJ/kg·°C)	Carbohydrate	$\hat{C}_p = 1.5488 + 1.9625 \times 10^{-3} T - 5.9399 \times 10^{-6} T^2$
	Ash	$\hat{C}_p = 1.0926 + 1.8896 \times 10^{-3} T - 3.6817 \times 10^{-6} T^2$
	Fiber	$\hat{C}_p = 1.8459 + 1.8306 \times 10^{-3} T - 4.6509 \times 10^{-6} T^2$
	Fat	$\hat{C}_p = 1.9842 + 1.4733 \times 10^{-3} T - 4.8008 \times 10^{-6} T^2$
	Protein	$\hat{C}_p = 2.0082 + 1.2089 \times 10^{-3} T - 1.3129 \times 10^{-6} T^2$

Source: Choi and Okos (1986b).

Table 2.2: Equations for calculating thermal properties of water and ice (Ibarz et al., 2003).

	Temperature Functions[a]	
Water	$\lambda = 0.57109 + 1.7625 \times 10^{-3}\, T - 6.7036 \times 10^{-6}\, T^2$	(W/m·°C)
	$\alpha_A = [0.13168 + 6.2477 \times 10^{-4}\, T - 2.4022 \times 10^{-6}\, T^2].10^{-6}$	(m^2/s)
	$\rho_A = 997.18 + 3.1439 \times 10^{-3}\, T - 3.7574 \times 10^{-3}\, T^2$	(kg/m^3)
	$\hat{C}_{PA1} = 4.0817 - 5.3062 \times 10^{-3}\, T + 9.9516 \times 10^{-4}\, T^2$	(kJ/kg·°C)
	$\hat{C}_{PA2} = 4.1762 - 9.0864 \times 10^{-5}\, T + 5.4731 \times 10^{-6}\, T^2$	(kJ/kg·°C)
Ice	$\lambda_H = 2.2196 - 6.2489 \times 10^{-3}\, T + 1.0154 \times 10^{-4}\, T^2$	(W/m·°C)
	$\alpha_H = [1.1756 - 6.0833 \times 10^{-3}\, T + 9.5037 \times 10^{-5}\, T^2] \times 10^{-6}$	(m^2/s)
	$\rho_H = 916.89 - 0.13071\, T$	(kg/m^3)
	$\hat{C}_{PH} = 2.0623 + 6.0769 \times 10^{-3}\, T$	(kJ/kg·°C)

[a] $\hat{C}_{PA1}$ = For a temperature range between −40 and 0°C.
$\hat{C}_{PA2}$ = For a temperature range between 0 and 150°C.

Source: Choi and Okos (1986b).

2.7. Questions and tasks

- Determine the content of water, carbohydrates, fats, proteins and ash in the sample.
- Calculate the energy value of the sample and the energy fractions of the individual components in the sample.
- Determine the density, thermal conductivity, specific heat and thermal diffusivity of the sample at 20, 25 and 30°C.

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