Food Process Quality Control Laboratory (S352004)
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Work no. 1b

Spectroscopic analysis of chocolate and products with cocoa powder

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The aim: Meet the measurement and interpretation of vibrational spectra of products containing chocolate and cocoa powder.

Work: Analyze the unknown samples of chocolate or other cocoa products containing components using FTIR and FT NIR spectra measured on the device Nicolet 6700 (Thermo Scientific, USA) to try to determine the differences in the composition of these samples on the basis of interpretation of spectra.

Theoretical part:

Chocolates are made from cocoa mass, cocoa butter and sucrose. Thus they contain non-fat cocoa solids, cocoa butter and sucrose. Milk and white chocolate also contains milk powder.Certain types of chocolate can be part of the cocoa butter (max 5%), replacement of special vegetable fat. Currently, many manufactured products that have a formula consisting of cocoa powder, specialty vegetable fats, or sucrose and powdered milk. Milk flavor is often supported by the addition of whey.

Infrared spectroscopy is based on the ability of substances to absorb electromagnetic radiation in the infrared (4000 - 400 cm 1). IR absorption of light quantum leads to vibration of the individual bonds and groups in the molecule. IR spectrum of substances containing individual bands corresponding to vibrational transitions, each bond or group usually has several absorption bands. Distinguish the valence (ν) and deformation (δ, γ) vibrations:

- valence, ν – changes mainly bond length
- deformation surface, δ (shuttle ρ, scissors δ) – change the angle between bonds
- deformation out of surface, γ (fan ω, twisting τ) – one atomvibrates out of the surface

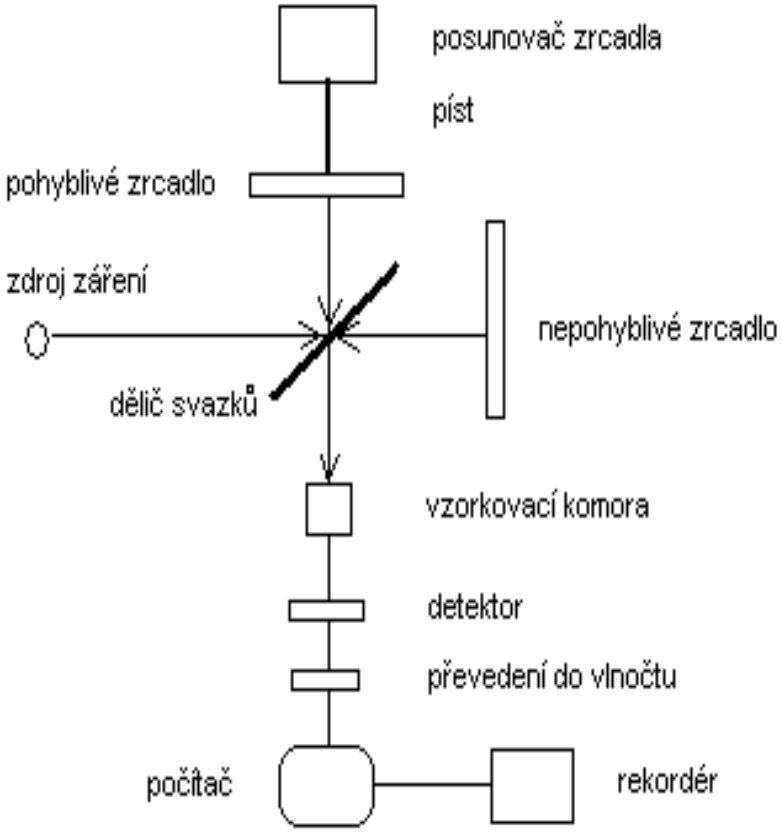
Dispersive infrared spectroscopy is used to measure the spectra of monochromatic light obtained by dividing the light beam is broken down into a grid or a prism into its individual color components, respectively. wavelengths. A device generating the monochromatic beam is called a monochromator. When actually measuring it by shooting through a mirror monochromatic beam of variable wavelength pattern, with which it reacts and the response is recorded as the IR spectrum, as the dependence of absorbance (transmittance) in the wave number. Dispersive infrared spectrometer can be single or Double beam. Dispersive spectrometers disadvantage is the large number of moving parts, low speed recording of the spectrum, a small optical transmission spectrometer stray light interference, lack of internal calibration and sample heating.

At present, the classical method of measuring waived, since this method is too slow.Frequently used method is now IR spectroscopy Furierovou transform (FT-IR). Instead of monochromatic light is used here polychromatic beam of light. The device works on the principle skenujícího Michelson interferometer. Custom interferometer consists of two mutually perpendicular plane mirrors, fixed and movable, and the beam splitters. Beam hits the divider, which is divided into two equal parts, which act to separate the two mirrors, reflect on them and dividing again recombine. The output of the beam splitters is so polychromatic beam interferovaného light that strikes the sample (see Figure 1). The result of interaction with the sample beam is interferogram, which is the sum of all functions kosinových wavelengths that passed interferometer. Interferogram is not applicable to the interpretation and so it is necessary to convert the infrared spectrum using the Fourier transform (FT). Given that FT is a mathematical method, you must have for interferogram processing computer with appropriate software. The advantage of FT-IR spectrometer lies in the fact that the measurement does not take long, as in conventional spectroscopy, thanks to the software spectrum obtained directly, if you increase the number of scans will provide greater accuracy.

IR spectra of samples in a different state can be measured by different techniques. Each technique requires special conditions. Cells used to measure infrared spectra of gaseous and liquid samples must be of a material which is permeable to infrared radiation material such as halides of some metals, especially alkali. When measuring solutions is necessary to ensure that the solvent used was permeable to infrared radiation in the desired area. The water appears to be inappropriate in this medium because it absorbs infrared radiation in several areas.

Solid samples, which may not be dissolved in a suitable solvent or can not be modified into a thin layer of a suitable processing (melting, molding or preparing the self-supporting films), are usually governed by nujolové suspensions or KBr tablets. When nujolové technique, the sample is mixed with paraffin oil (nujolem) and pulverized in an agate mortar to a thick slurry, which is then measured in the capillary layer between two salt windows. Tablets of the sample in potassium bromide is prepared from a mixture of sample and dried KBr (ratio 1-5: 100).This mixture is adjusted to achieve the finest consistency, and then pressed under great pressure to the transparent tablet.
There are infrared spectra measurement techniques that allow analysis of complex samples such as powders, aqueous solutions or mixtures. Diffuse reflectance technique (DRIFT, diffuse reflectance infrared Fourier transform) is measured primarily powdered samples, which can not be exposed to high pressure between the analyte and the matrix leads to ion exchange, etc.

Measurement is simple: a homogeneous sample turns out to focus radiation from the source.This radiation particles in the sample partly reflects and partly penetrates, the absorbed radiation is either completely absorbed or only weakened. Radiation reflected from the sample surface and the radiation from the sample is protruding from the space above the sample carried away with the mirror on the detector. Here's radiation is divided into several components and mathematically converted by Kubelková-Munk equation for the dependence of the log (1 / R) where R is the reflectance (%), and concentration of the analyte. The combination of FT-IR spectroscopy with attenuated total reflectance (ATR, attenuated total reflectance) to achieve greater excellence and solve problems arising in the diffuse reflectance (high water absorption, poor permeability in the middle infrared region). ATR is based on the transmission of infrared radiation, a crystal, which is in contact with the sample.At each reflection wave penetrates into the space behind the crystal depths of the order of several micrometers. The sample, which is in contact with the contact surfaces, can interact with a beam of infrared rays and provide absorption spectrum.



Obr. 1: Schema of Michelson interferometer

Experimental work

Preparation of sample and measuring FTIR spectra – KBr tablets

Weigh approximately 5 mg of the sample and mix with 100 mg of KBr in an agate mortar. Pour the mixture into the molding attachment for KBr tablets and put the hand press. Push lever and remove the adapter with the tablet. Place the tube with pure KBr in the sample chamberdevice. Of measured background. Replace the adapter and the range of measured tabletsample. Save the spectrum and make the necessary adjustments (smoothing, correction of CO_2 and water vapor, baseline correction). Print and mark the position spectrum band and their assignment on the basis of wave numbers table assignment.

Preparation of sample and measuring FTIR spectra – HATR

Of measured background (no sample) and ready to sample measurement. Place the samplein the attachment area (the crystal) and press down until the screen shows a significant range.Range of measured sample, save it and make the necessary adjustments (stroking, ATRcorrection and baseline correction). Print and mark the position spectrum band and itsassignment on the basis of wave numbers table assignment.

Preparation of sample and measuring FTIR spectra – difuse reflectance

Weigh approximately 5 mg of the sample and mix with 100 mg of KBr in an agate mortar. Pourmixture into bits for measurement. Place the tube with pure KBr in the sample chamber device.Of measured background. Replace the adapter and the range of measured sample. Print and mark the position spectrum band and their assignment on the basis of wave numbers tableassignment.

Preparation of sample and measuring FT NIR spectra – difuse reflectance

Of measured background (spektralonový standard). Place the sample in the attachment areaand push it above. Range of measured sample and save it. Print and mark the positionspectrum band and their assignment to the assignment of wave numbers in table NIR region.