Effect of fat composition on some physico-chemical parameters and sensorial evaluation of dark chocolate

EVA VÍTOVÁ – BLANKA LOUPANCOVÁ – HANA ŠTOUDKOVÁ – IVANA MACKŮ – JANA ZEMANOVÁ – LIBOR BABÁK

Summary

The aim of this work was to compare several physical, chemical and organoleptic properties of three types of dark chocolate with different contents of vegetable fat to identify if partial or total replacement of cocoa butter influences chosen parameters. Aroma compounds were determined by the solid-phase microextraction coupled to gas chromatography, fatty acids as methyl esters using gas chromatography, flavour and colour were evaluated sensorially, and texture by both instrumental and sensory analyses. Partial replacement of cocoa butter (up to 5% allowed by the European legislation) had only negligible effect on the monitored properties, differences between the samples were statistically insignificant ($P < 0.05$). However, total replacement of cocoa butter (as in various chocolate imitations) significantly ($P < 0.05$) influenced the properties. All the monitored physical and chemical parameters were different, high concentration (341.17 ± 13.49 mg.g^{-1}) of elaidic acid was found here. The most evident difference was in organoleptic properties, evaluated as less expressive, atypical for good chocolate, taste and aroma with strong oleic note, which is negatively perceived by consumers.

Keywords

chocolate; vegetable fat; fatty acids; aroma; solid-phase microextraction; gas chromatography; sensory analysis

Chocolate is one of the most popular foods all over the world because of its organoleptic properties. It has sweet taste due to the large amount of sugar (about 50%, according to the type), pleasant aroma caused by many aroma compounds and special texture, which is solid, brittle, but melts at body temperature, thereby contributing to the overall pleasurable, cooling effect in the mouth. The desired physical properties of chocolate, i.e. texture, melting behaviour, gloss, snap etc. are functionally related to the crystal network of cocoa butter consisting of triacylglycerols (TAG) [1, 2]. Tempering is necessary to obtain these properties. It ensures formation of fine crystals in the desired form (β-modification), otherwise cocoa butter tends to crystallize in rather coarse crystals perceivable in mouth. Well tempered chocolate has fine, completely homogenous structure, fine dissolving taste, solid consistence and fracture, gloss surface [3].

High price, fluctuation in supply and demand, as well as inadequate quality of certain harvests evoked the need to substitute cocoa butter, which lead to the development of the cocoa butter alternatives. There is no other naturally occurring fat with the same physical properties as cocoa butter. Thus, all possible alternatives are made by blending and/or modifying fats. They can be obtained from fruits and seeds, or produced specifically by chemical or enzymatic pre-esterification. The major methods for modification of fats are fractionated crystallization and interesterification [1, 4]. Novel sources to produce cocoa butter alternatives have been sought for, using alternative tropical fats, microbial lipids or (bio)chemical processing of TAG. However, none of these alternatives has found general acceptance [2].

Cocoa butter alternatives must have similar chemical and physical properties to cocoa butter, in particular the melting behaviour, in order to

Eva Vítová, Blanka Loupancová, Hana Štoudková, Jana Zemanová, Libor Babák, Department of Food Chemistry and Biotechnology, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, CZ – 612 00 Brno, Czech Republic.
Ivana Macků, Department of Food Engineering, Faculty of Technology, Tomas Bata University, Náměstí T. G. Masaryka 588, CZ – 760 01 Zlín, Czech Republic.

Correspondence author:
Eva Vítová, tel: +420 541149475, fax: +420 541211697, e-mail: evavitova@post.cz

achieve the same "mouth feeling", and must not affect crystallization, otherwise textural and visual defects (fat bloom) may develop. Palm oil fractions and other vegetable fats of tropical origin (shea, sal, illipé) form the basis for manufacturing of cocoa butter alternatives. These may be used for partial or total replacement of cocoa butter. Certain of them can even improve some quality-related features (resistance to bloom, increased milk fat tolerance etc.). However, the main reason for their use is the economic advantage [1, 2, 5].

Because the addition of vegetable fat can influence flavour and other organoleptic properties of chocolate, current European legislation (Directive 2000/36/EC) allows their addition only up to a level of 5% of the product weight, provided that it is correctly indicated on the label [2, 6].

Due to their similarity to genuine cocoa butter, cocoa butter alternatives are difficult to detect. Several authors, e.g. ČOPÍKOVÁ et al. [3], ULBERTH et al. [2] and LIPP et al. [7] described methods suitable for determination of cocoa butter alternatives. They can be detected either by determining the fatty acids and/or TAG profile, by analysis of minor fat constituents (sterols, sterol degradation products, terpenes), or by spectroscopic and thermoanalytical techniques [4]. Several authors tried to develop a method for the detection and even quantification of cocoa butter alternatives in chocolate, e.g. BOHAČENKO et al. [8], BUCHGRABER et al. [9–11], GUYON et al. [12], DIONISI et al. [13].

Flavour (aroma and taste) is very important property of chocolate. It is formed during technological process, the main cocoa flavour components are alcohols, ethers, hydrocarbons, furans, thiazoles, pyridines, acids, esters, aldehydes, ketones, imines, amines, oxazoles, pyrazines and pyrroles [14]. Colour of chocolate is created mainly during the roasting process. Oxidation and polymerization of polyphenols, degradation of proteins, Maillard reaction and dextrinization of starch yield the desired brown pigments [15].

The aim of this work was to compare several physical, chemical and organoleptic properties of three types of dark chocolate with different contents of vegetable fat. We attempted to identify if partial or total replacement of cocoa butter by vegetable fat influences these properties and, consequently, also the acceptance of the product by the consumers. Aroma compounds were determined by solid-phase microextraction coupled to gas chromatography (SPME-GC), fatty acids as methyl esters using GC, flavour and colour were evaluated sensorially, texture by both instrumental and sensory analyses.

MATERIALS AND METHODS

Chemicals

The following chemicals were used as standards: benzothiazol, decan-2-one, dimethyl disulfide, dimethyl sulfide, dimethyl trisulfide, heptadecane, heptadecan-1-ol, heptadecan-2-ol, heptanal, hexadecan-2-ol, hexanal, pentadecane, phenylacetaldehyde, 8-nonen-2-one, phenylethyl-acetate, pentyl-benzoate (Sigma-Aldrich, Deisenhofen, Germany), acetaldehyde, propionaldehyde, butan-2,3-diol, decanol, ethanal, ethyl-butyrate, ethyl-caprylate, ethyl-caprinate, hexan-1-ol, 3-hydroxybutan-2-one, nonan-2-one, phenylethanol, phenylmethanol, propanal, pentan-2-one, undecan-2-one, heptan-2-on (Merck, Darmstadt, Germany), acetic acid, propanoic acid, butanoic acid, acetone, butan-2-one, methanol, propan-1-ol, propan-2-ol, butan-2-ol, butan-1-ol, pentan-1-ol, pentan-2-ol, octan-1-ol, nonan-2-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, propan-2-one, methyl-acetate, ethyl-acetate, propyl-acetate, butyl-acetate (Lachema, Brno, Czech Republic), benzaldehyde, dodecan-1-ol, ethanol, heptane, heptan-2-ol (J. T. Baker, Deventer, Netherlands), butan-2,3-dione, 3-methylbutan-1-al, oct-1-en-3-ol (Fluka, Buchs, Switzerland). All the chemicals were of chemically pure grade.

A mixture of 37 methyl ester standards of fatty acids (Supelco 37 Component FAME mix; Supelco, Bellefonte, Pennsylvania, USA) was used for analysis of fatty acids.

Samples

Three types of dark chocolate were tested in this work: plain chocolate (30.5% fat; without addition of vegetable fat), chocolate for cooking (27% fat; addition of vegetable fat up to 5%), chocolate glaze (34% fat; cocoa butter completely replaced by vegetable fat). Four batches of chocolate samples were evaluated. All samples were purchased from the Czech retail market.

SPME-GC analysis of aroma compounds

Grated chocolate (1 g) was analysed.

SPME conditions: the fibre CAR/PDMS 85 μm (Supelco), equilibration time 30 min, extraction for 20 min at 35 °C.

GC conditions: Gas chromatograph TRACE GC (ThermoQuest, Milan, Italy) equipped with a flame ionization detector (FID) and split/splitless injection port, DB-WAX capillary column (30 m × 0.32 mm × 0.5 μm; J&W Scientific, Folsom, California, USA). The injector – 250 °C, splitless mode, the desorption time 5 min, linear purge closed for 5 min. Temperature

of the detector was 220 °C. The flow of the carrier gas (N_2) was 0.9 ml.min^{-1}. The oven temperature programme: 40 °C for 1 min, heating at 5 °C.min^{-1}, 200 °C for 7 min.

The identification was carried out by GC-MS and confirmed by comparison of the retention times with standards. The mass spectra were compared with standard mass spectra provided by the database of the equipment. The method of standard addition was used for quantification. The sample preparation and validation of this method was described in detail in our earlier studies [16].

Gas chromatography and mass spectrometry (GC-MS)

Gas chromatograph GC 8000 (Carlo Erba, Milan, Italy) coupled to a MS TRIO 1000 (Fisons Instruments, Valencia, California, USA) was used. The ionizer temperature was set to 150 °C, electron impact mode was used, with electron energy of 70 eV. The carrier gas was He with a head pressure of 150 kPa, the GC column and other operating parameters were the same as described above.

GC analysis of fatty acids

The fat was extracted from chocolate sample (5 g) according to ČSN 56 0146 part 4 [17] using Soxhlet extraction with petroleum ether. Fatty acids were analysed as methyl esters, methanol esterification method with potassium hydroxide catalysis was used; the preparation of methyl esters was described in detail in our earlier study [18]. GC conditions: Gas chromatograph TRACE GC, capillary column SP 2560 (100 m × 0,25 mm × 0,2 μm; Supelco). The injector temperature was 250 °C, splitless mode was used, the desorption time was 5 min, linear purge was closed for 5 min. The temperature of FID was 220 °C. Flow of the carrier gas (N_2) was 1.2 ml.min^{-1}. The oven temperature programme: 60 °C for 2 min, heating at 10 °C.min^{-1}, 220 °C for 20 min.

Fatty acids methyl esters were identified by comparison of their retention times with those of standards. The method of standard addition was used for quantification.

Sensory analysis

Sensory evaluations were performed in a specialized testing room equipped with individual booths. For the sensory analysis, chocolate samples were cut in identical cubes (~10 g). Unsalted rolls were served to remove any aftertaste.

Sensory attributes, i.e. appearance and colour, taste and aroma, texture and overall acceptability were evaluated using 5-point hedonic scale (1 – unsatisfactory, 2 – less good, 3 – good, 4 – very good, 5 – excellent). Finally, panelists were asked to rank samples for overall palatability (ranking test). The test panel consisted of 14–16 persons selected from students and staff of the Faculty of Chemistry, Brno University of Technology, who were initially screened to establish their ability to recognize the primary taste stimuli.

Texture measurement

The texture was determined using an Instron 5544 instrument (Instron, High Wycombe, United Kingdom) connected to a computer (software Series IX) equipped with a rectangular attachment for cutting. The velocity of the head with the attachment was 100 mm.min^{-1}. The measurements were taken for determining maximum shear force (F_{max}) necessary to cut the chocolate sample (8 mm height).

Statistical analysis

The results of chemical and instrumental analyses were statistically treated using MS Excel 2003 (Microsoft Corporation, Redmond, Washington, USA), the results are expressed as mean ± standard deviation (n = 12).

The results of chemical and sensory analyses were statistically evaluated by means of Kruskall-Wallis test. For these statistical evaluations, Unistat version 5.5 (Unistat, London, United Kingdom) was used.

RESULTS AND DISCUSSION

SPME-GC analysis of aroma compounds

Chocolate taste and aroma is directly connected to the chemical composition, especially to the contents of volatile aroma compounds. The most significant flavour-impact substances in cocoa derivatives are N- and O-containing heterocyclic compounds generated during roasting, which are products of the Maillard reaction. The most important are alkylpyrazines, in terms of contribution to chocolate sensory characteristics [19]. Our aim was to monitor alcohols, aldehydes, ketones, acids, esters, several sulfur and nitrogen compounds as minor aroma compounds of chocolate. SPME-GC was used for the analysis of volatile compounds. This method is simple, rapid and very mild to the matrix, so it is suitable for the characterization of the food aroma and for comparison with sensory analysis. Several authors also applied SPME-GC for determination of volatile compounds in cocoa products [19, 20].

In total, 40 volatiles were identified in chocolate samples: 17 alcohols (methanol, ethanol,

propan-1-ol, butan-1-ol, butan-2-ol, pentan-1-ol, pentan-2-ol, hexan-1-ol, heptan-2-ol, octan-1-ol, nonan-2-ol, decanol, 3-methylbutan-1-ol, butan-2,3-diol, oct-1-en-3-ol, phenylethanol and phenylmethanol); 6 aldehydes (acetaldehyde, benzaldehyde, heptanal, hexanal, phenylacetaldehyde and 3-methylbutan-1-al); 7 ketones (acetone, butan-2-one, pentan-2-one, heptan-2-on, nonan-2-one, 3-hydroxybutan-2-one and butan-2,3-dione); 3 acids (acetic, propanoic and butanoic acids) and 7 esters (ethyl-acetate, butyl-acetate, ethyl-butyrate, ethyl-caprylate, ethyl-caprinate, phenylethyl-acetate and propyl-acetate).

In plain chocolate, 40 volatiles were identified. The most abundant were ethanol (118.65 ± ± 6.04) $\mu g.g^{-1}$, propan-1-ol (95.37 ± 11.78) $\mu g.g^{-1}$, butan-2,3-diol (543.30 ± 71.72) $\mu g.g^{-1}$ and phenylacetaldehyde (52.24 ± 4.27) $\mu g.g^{-1}$.

In chocolate for cooking, 37 volatiles were identified. The most abundant were methanol (18.89 ± 1.14) $\mu g.g^{-1}$, ethanol (139.67 ± 13.14) $\mu g.g^{-1}$, propan-1-ol (74.14 ± 8.55) $\mu g.g^{-1}$, butan-2,3-diol (405.65 ± 14.13) $\mu g.g^{-1}$, acetic acid (165.99 ± 20.48) $\mu g.g^{-1}$, acetone (17.35 ± 2.55) $\mu g.g^{-1}$, acetaldehyde (22.56 ± 2.50) $\mu g.g^{-1}$ and phenylacetaldehyde (38.25 ± 3.35) $\mu g.g^{-1}$.

In chocolate glaze 36 volatiles were identified, the most abundant were methanol (73.77 ± 5.86) $\mu g.g^{-1}$, ethanol (174.78 ± 2.41) $\mu g.g^{-1}$, propan-1-ol (68.74 ± 5.97) $\mu g.g^{-1}$, butan-2,3-diol (754.82 ± ± 58.31) $\mu g.g^{-1}$, acetone (21.22 ± 1.75) $\mu g.g^{-1}$, butyl-acetate (18.66 ± 1.54) $\mu g.g^{-1}$ and phenylacetaldehyde (19.09 ± 0.94) $\mu g.g^{-1}$.

Several authors analysed volatile flavour components of cocoa beans, cocoa and chocolate, and several hundreds volatile compounds have been identified [14, 21, 22]. However, no attempt was made to include volatile compounds for characterization of the vegetable fats in mixture with cocoa butter. According to Lipp et al. [1], it is not useful for several reasons: the flavour typical for cocoa butter alters significantly with climate, seasonal variations, country of origin etc., the vegetable fats normally show no flavour at all and, moreover, flavour constituents are easy to remove during desodoration, which is widely applied in the industry.

Nevertheless, in this study, as can be seen in Fig. 1., the contents of aroma compounds differs in single chocolate samples, although it is not necessarily caused by the replacement of cocoa butter by vegetable fat. The cocoa aroma components come mainly from sugars and proteins.

Aroma profiles of plain chocolate and chocolate for cooking were similar, only the content of short-chain fatty acids was significantly ($P < 0.05$) higher in chocolate for cooking. The aroma profile of chocolate glaze was rather different, it contained significantly ($P < 0.05$) higher amounts of alcohols and esters and, conversely, significantly ($P < 0.05$) lower amounts of aldehydes. All types of chocolate contained markedly high concentrations of alcohols in comparison with other chemical groups of compounds.

GC analysis of fatty acids

The fat phase significantly influences the taste and texture of chocolate. Cocoa butter contains substantial quantities of 2-oleyl glycerides of palmitic and stearic acids, which are mainly responsible for the crystallization and melting characteristics. Consequently, the predominant fatty acids in cocoa butter are stearic, palmitic and oleic acids.

Analysis of fatty acids is a well-known and widely applied technique for checking the purity of fats and oils. Acetic, propanoic and butanoic acids were analysed directly using SPME-GC owing to their volatility and data on them are given in previous section. The other fatty acids were analysed in the extracted fat as methyl esters using GC. This method is simple, its only disadvantage is a long

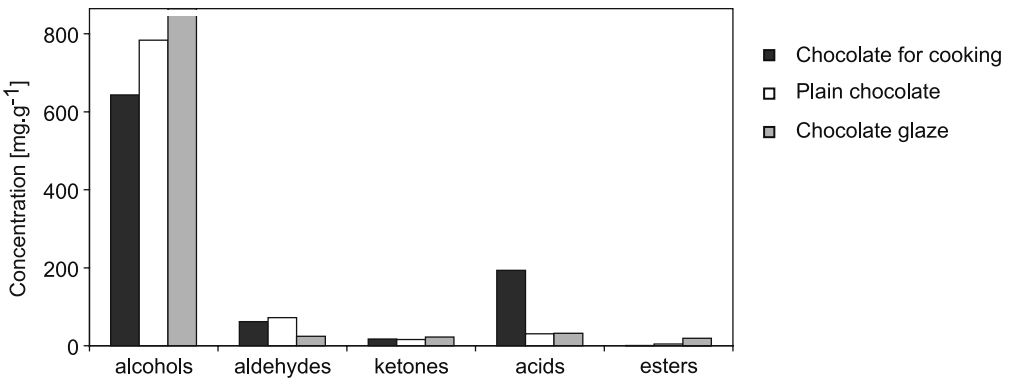

Fig. 1. Comparison of groups of aroma compounds in three tested types of chocolate.

Tab. 1. Fatty acids identified in chocolate samples.

	Chocolate for cooking	Plain chocolate	Chocolate glaze
Fatty acid	Concentration [mg.g^{-1}]		
caprylic	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
capric	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
lauric	0.03 ± 0.00	0.11 ± 0.01	0.17 ± 0.01
myristic	5.28 ± 0.02	3.66 ± 0.03	13.12 ± 0.14
myristoleic	0.01 ± 0.00	0.01 ± 0.00	0.00
pentadecanoic	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
palmitic	321.03 ± 9.41	293.82 ± 8.46	385.62 ± 12.94
palmitoleic	0.14 ± 0.01	0.24 ± 0.02	0.04 ± 0.00
heptadecanoic	0.13 ± 0.01	0.34 ± 0.03	0.07 ± 0.01
stearic	309.54 ± 14.89	336.57 ± 12.51	86.29 ± 1.44
elaidic	0.00	0.00	341.17 ± 13.49
oleic	309.48 ± 11.38	314.91 ± 10.38	143.02 ± 6.16
linoleic	35.20 ± 0.23	31.68 ± 1.16	12.39 ± 0.09
arachidic	8.96 ± 0.05	8.68 ± 0.06	4.19 ± 0.03
linolenic	0.17 ± 0.01	0.29 ± 0.02	0.08 ± 0.01
behenic	0.12 ± 0.01	0.35 ± 0.02	0.07 ± 0.01

The results are expressed as mg.g^{-1} of fat.

extraction time. The method was validated for cheeses in our earlier studies [18] and its application for chocolate analysis was confirmed.

In total, 16 fatty acids were identified in chocolate samples (Tab. 1). Main components of the plain chocolate were myristic, palmitic, stearic, oleic, linoleic and arachidic acids. Main components of chocolate for cooking were myristic, palmitic, stearic, oleic, linoleic and arachidic acids. Main components of chocolate were myristic, palmitic, stearic, elaidic, oleic, linoleic and arachidic acids.

As can be seen in Fig. 2, there were no significant ($P < 0.05$) differences in the contents of most important fatty acids between plain chocolate and chocolate for cooking. However, the composition of main fatty acids in chocolate glaze was different. The contents of stearic, oleic and linoleic acids were significantly ($P < 0.05$) lower and, conversely, the contents of myristic and palmitic acids were significantly ($P < 0.05$) higher. Moreover, high concentration of elaidic acid was found here. This *trans*-fatty acids was present only in chocolate glaze. Cocoa butter has naturally a very low content of *trans*-fatty acids, which are formed during hardening of fats and oils that is based on hydrogenation of double bonds. This fact could be used for the detection of vegetable fats in chocolate [3, 7].

Sensory analysis

According to the Czech legislation, taste and aroma of chocolate should be pleasant, aromatic and after the used raw material. It should be well-

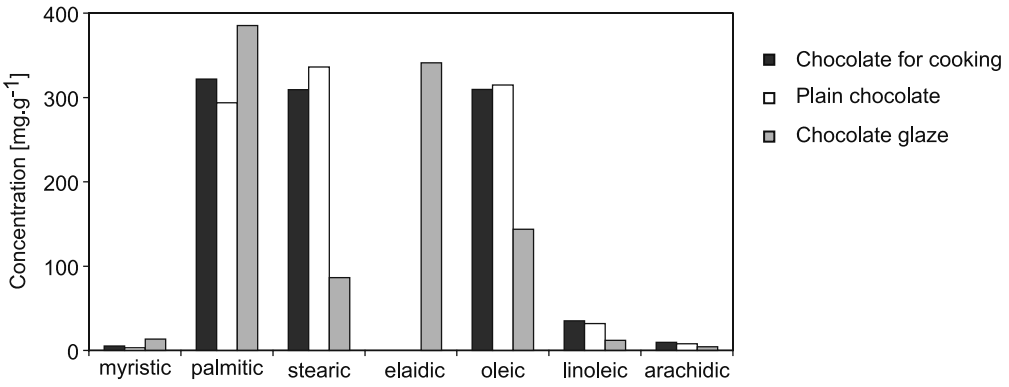

Fig. 2. Comparison of most important fatty acids in chocolates.

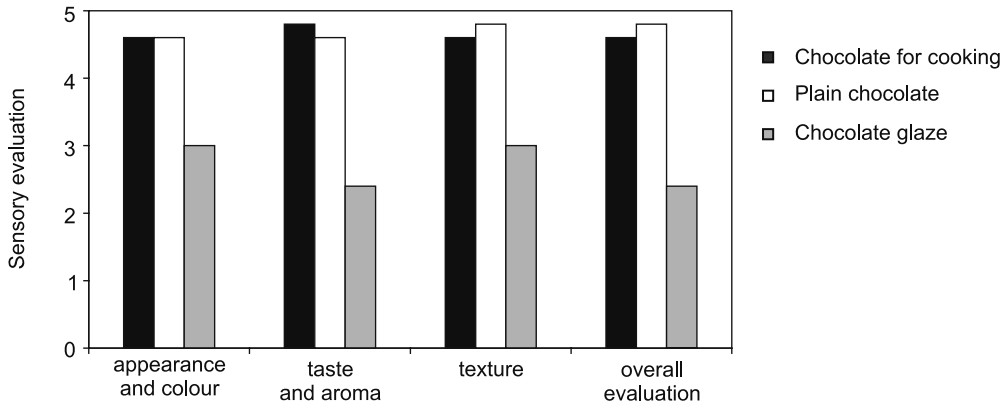

Fig. 3. Sensory evaluation of organoleptic properties of chocolates.
Legend: 1 – unsatisfactory, 2 – less good, 3 – good, 4 – very good, 5 – excellent.

dissolving in mouth, solid, of a brittle texture and gloss surface. Evaluation of appearance, colour, taste, aroma and texture using 5-point hedonic scale was used for sensory evaluation of chocolate samples, where "excellent" corresponded to legislation requirements and "unsatisfactory" meant marked perceivable defects. The results are graphically expressed in Fig. 3. Plain chocolate and chocolate for cooking were evaluated very similarly, mostly very good or excellent in all signs, no statistical difference ($P < 0.05$) was found.

The colour of chocolate glaze was less expressive, appearance was rather less glossy, in sum evaluated as less good or good, also significantly worse ($P < 0.05$) than that of other chocolate. The taste and aroma of chocolate glaze was evaluated as less characteristic, less good, also significantly worse ($P < 0.05$) than that of other chocolate. Relatively strong oleic perception was described here. Texture of chocolate glaze was described as softer, less brittle, which is not typical for good chocolate, also texture was significantly worse ($P < 0.05$) than that of other chocolates. The most evident difference between chocolate glaze and other chocolates was in taste and aroma, significantly influencing the overall evaluation of sensory quality. In total, plain chocolate was evaluated as the best, chocolate glaze as the worst (Fig. 3).

In terms of ranking, test panelists evaluated overall palatability of chocolate samples. The results agreed with conclusions of scale tests. According to the sum of ranks, chocolate for cooking was the most tasty, chocolate glaze was significantly ($P < 0.05$) the worst. The difference between chocolate glaze and chocolate for cooking was described as obvious by the panelists. The difference between plain chocolate and chocolate for cooking was described as negligible by the panelists, there was no statistical difference ($P < 0.05$).

To summarize, although the composition of individual types of chocolate was rather different, panelists were unable to distinguish sensorially plain chocolate and chocolate for cooking. Legislation allows the addition of vegetable fats to chocolate up to 5%. This fat probably does not influence overall sensory quality and palatability of chocolate and hence does not change its acceptance by the consumers. The taste, aroma and overall palatability of chocolate for cooking were even evaluated better than those of plain chocolate. However, total replacement of cocoa butter by vegetable fat significantly influenced the sensory quality of chocolate. Chocolate glaze was evaluated as the worst in all categories, the differences from other two chocolate were perceived as strong, obvious. Although chocolate glaze looked like real chocolate, its taste was not so delicious. Thus it is not suitable for direct consumption. However, chocolate glaze is suitable as a component of chocolate sweets, where its unpleasant oleic off-flavour is not so marked.

Instrumental texture measurement

Texture generally includes mechanical, geometric and surface properties of products. It can be objectively measured using various methods, which are mostly based on the measurement of resistance of food to stress [23]. The rheological properties are most often measured to describe the texture of chocolate and related cocoa products [24–29].

Texture is, next to colour and flavour, an important criterion of sensory evaluation of food and its acceptation by consumer. It even contributes to the flavour perception, because the texture

traps the flavour. The taste of chocolate depends on flavour compounds released by chewing to the mouth and nose, perceived texture is a function of the way in which the material melts and breaks up in the mouth.

In our study, the texture of chocolate samples was measured in Instron 5544 using Warner-Bratzler test, which simulates the first bite. F_{max} represents the hardness of the sample. There was no significant difference in hardness of plain chocolate (50.53 ± 2.92) N and chocolate for cooking (46.12 ± 6.88) N, while chocolate glaze was objectively softer (29.30 ± 1.32) N. This is in good accordance with sensory evaluation, wher the texture of chocolate glaze was described as softer, less brittle, atypical for good chocolate. So the replacement of cocoa butter in chocolate could influence its mechanical properties.

CONCLUSIONS

For several reasons, cocoa butter in chocolate is often partly or fully substituted by vegetable fat. The partial replacement of cocoa butter (up to 5% is allowed by the European legislation) had negligible effect on the monitored properties, so it probably did not influence the overall sensory quality and palatability of chocolate, and hence did not change the acceptance by the consumers.

However, total replacement of cocoa butter, in chocolate glaze in this study, significantly influenced composition and properties of chocolate. All the monitored physical and chemical parameters were affected. The only *trans*-fatty acids, elaidic, was present here in a considerably high concentration. The most evident difference between chocolate glaze and other chocolate types was in organoleptic properties, evaluated as less expressive, atypical for good chocolate, taste and aroma with a strong oleic note, which altogether negatively influenced the acceptance by the consumers.

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