LWT - Food Science and Technology 154 (2022) 112669

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Physical characterization of the milk chocolate using whey powder

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ARTICLE INFO

Keywords:
Milk chocolate
Whey powder
Textural properties
Particle size
DSC

ABSTRACT

In this study it was found that the complex microstructure of chocolate was modified by the addition of whey into original milk chocolate. A decreasing particle size diameter of the dispersed cocoa fat particles with increasing whey content followed by dynamic light scattering measurements was confirmed. Changes of the chocolate fat crystallinity through disrupting the highly ordered cocoa fat dense crystal aggregates to the less developed ones deformed by the inclusion of whey particles into the complex chocolate mass were simultaneously confirmed. These morphology changes affected the thermal and rheological behaviour of the studied samples by decreasing the melting peak temperature as well as Casson's plastic viscosity with increasing whey content. Furthermore, a gradual decrease of the hardness, thus reflecting the plasticising effect of the whey particles on the complex crystalline structure of the chocolate, was observed. The latter fact was also confirmed by the decreased acoustic emissions during chocolate breakage, thus indicating the change of the fracture process from brittle to more ductile.

1. Introduction

Milk chocolate is composed of milk components added as solid particles (milk powder and whey powder) refined with cocoa liquor and sugar, which are dispersed within the fat phase (Atik, Boluk, Toker, Palabiyik, & Konar, 2020). The main fat component, cocoa butter, holds all the solid sugar and cocoa particles together (Lapčík, Lapčíková, Žižková, Peng, & Vojteková, 2017). Additional ingredients, such as whey powder, can substantially modify the functional properties, sensory perception and overall quality of chocolate products. Whey is becoming increasingly popular and worthwhile for its nutritional quality and health benefits, such as the provision of amino acids essential for muscle synthesis (Bull et al., 2017; Carter, Foegeding, & Drake, 2020). From a nutritional point of view, the function of whey reduces calories, fat content and chocolate sweetness. Whey additionally increases the rate of Maillard reaction during the conching and enables the caramelised flavour production in milk-powder-based chocolate. More specifically, whey powder with a low mineral content is able to avoid a salty flavour, which can often be detected in the chocolate. In general, the replacement of milk solids with whey powder optimises manufacturing costs by as much as 8%–14% (Bouzas & Hess, 2011, p. 501). According to EU standards, functional dairy ingredients may be used to formulate milk chocolate in addition to milk at a level not higher than 5 w.% of the total chocolate mass (Anonymous, 1998). In the USA, the FDA's identifying standard only permits the use of whey-based ingredients up to 5 w.% in white chocolate (Anonymous, 2004, section 163).

Particle size is an important factor of whey use in food and beverage products, respectively in chocolate. The distribution of particle size affects rheology and texture, which has a great influence on yield stress, plastic viscosity, hardness and other product parameters. Smaller particles improve the sensory properties of chocolate products, but surface area changes of particles in contact with fat phase leads to the increase of plastic viscosity and yield stress (Afoakwa, Paterson, Fowler, & Vieira, 2008).

As determined by previous studies, physico-chemical treatments have a basic effect on the particle size of whey influencing the functional properties and shelf-life of the final products. The character of chocolate

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https://doi.org/10.1016/j.lwt.2021.112669
Received 24 July 2021; Received in revised form 22 September 2021; Accepted 18 October 2021
Available online 19 October 2021

products is based on their specific particle size distribution, extent of particles dispersion or, on the other hand, particles aggregation, which can vary considerably in a relatively narrow temperature range. During processing, chocolate particle size is reduced and agglomerates broken, whereas lipid-coated particles are distributed through the continuous phase (Afoakwa, Paterson, Fowler, & Vieira, 2009; Bull et al., 2017; Carter et al., 2020).

Textural and rheological measurements are used to determine the effects of processing conditions on food texture. Chocolate texture is basically influenced by the triglyceride packing structure (polymorphs), microstructural properties, dispersed particles, particle size distribution, as well as by solid fat content and ratio of solid to liquid fat in a final product (Ostrowska-Ligęza et al., 2019).

Rheological properties of chocolate are dependent on the products' composition (fat content, particle size, etc.) and are affected by process conditions (refining, conching, tempering) and storage of the product (Afoakwa et al., 2008). The viscosity and yield stress of chocolate should particularly be taken into consideration as important parameters. Chocolate as a Bingham fluid has a yield stress, where a considerable amount of force is applied to begin the flow of the chocolate, which becomes thinner (less viscous) upon using higher force. Casson's model is conveniently used to determine chocolate flow properties, where Casson's yield stress is the energy required to start chocolate flow. Casson's plastic viscosity is related to the energy that is needed to keep the chocolate moving after it has begun to flow (Bahari & Akoh, 2018).

Chocolate as a complex system of ingredients is very difficult to analyse and relatively easy to falsify. For this reason, a sensitive tool able to determine the composition of chocolate is necessary. From a thermoanalytical point of view, differential scanning calorimetry (DSC) and thermogravimetry (TGA) represent the best options in the field of food analysis. DSC can be used in both isothermal and non-isothermal mode, providing information about crystallisation and oxidative stability, respectively. TGA is a simple, cost-effective method that can be employed to determine chocolate composition and consequently to control the quality of the final product (Dolatowska-Zebrowska, Ostrowska-Ligeza, Wirkowska-Wojdyla, Brys, & Gorska, 2019).

DSC is widely used to characterise melting profile changes in chocolate and measures the relative amount of each crystalline state. Cocoa butter can crystallise in VI polymorphic forms according to its TAG composition, from the least stable form sub-α or γ ($T_m \approx 19$ °C) to the most desirable form β_2 with a melting temperature of 32–34 °C (Ostrowska-Ligęza et al., 2019).

Acoustic analysis is a relatively cheap, fast, reliable and available technique that is applied in the food industry in order to determine the quality of agricultural and food products including crunchiness, crispness, firmness, water activity, etc. (Voong, Norton-Welch, Mills, & Norton, 2019). The acoustic properties of food products can be determined by destructive and non-destructive tests (passive and active methods). Acoustic methods are divided into methods of measuring sound emission, sound absorption and other methods of determining phase oscillation (Aboonajmi, Jahangiri, & Hassan-Beygi, 2015). For the right understanding of whey content effect on the functional properties of chocolate products, relevant parameters should be determined, notably particle size and thermal parameters, i.e., temperature peaks and enthalpy. It makes sense to know how average particle size and whey content influence the thermal stability of chocolate products, proteins denaturation, lactose crystallisation and lactose melting, enthalpy, etc.

The aim of this study is to examine the functional properties of milk chocolate with various whey powder contents. Particle size distribution can influence the overall physico-chemical characteristics of the whey chocolate system, which was characterised by dynamic light scattering and SEM analysis, as well as by rheological and acoustic measurements. The thermal behaviour of prepared samples was evaluated using differential scanning calorimetry (DSC) and thermogravimetry and related to ingredients of chocolate, especially melting fat and whey lactose.

2. Materials and methods

2.1. Milk chocolate production

The ingredients utilised for the production of the milk chocolate samples were: sucrose (Moravskoslezské cukrovary Ltd.; Czechia), cocoa butter (Svět Plodů Ltd.; Czechia), natural cocoa mass (Svět Plodů Ltd.; Czechia), skimmed milk powder (Moravia Lacto; Czechia) or whey powder (Mogador Ltd.; Czechia) and soy lecithin (Danisco Czech Republic; Czechia). The total weight of the produced milk chocolate samples ranged within the interval of 1025–1046 g per batch. Cocoa mass, cocoa butter, sucrose and dairy powder (whey or skimmed milk) were mixed according to product formulation (Table 1) and preheated to 50 °C using a Stephan UMC-5 (Stephan Machinery; Germany) equipped with indirect heating. The developed chocolate masses were then added to a chocolate melanger (Spectra 11 Melanger, India) for refining and conching (for a period of 20 h). Furthermore, soy lecithin was added during the last 30 min of conching. Tempering (Minitemper; Pavoni, Italy) of the developed samples followed thereafter. In addition, the milk chocolate samples were produced according to a protocol previously described by (Aaltonen, Kyto, Ylisjunttila-Huusko, & Outinen, 2020). After the tempering process, the chocolate samples were moulded. High density polyethylene moulds (25 mm wide, 100 mm long and 15 mm high) were applied to produce chocolate bars of approximately 25 g. The solid milk chocolate was demoulded and wrapped in aluminium foil and stored at (6.0 ± 0.5)°C until later analysis. A sample without whey powder addition was used as the control sample. Each formulation was prepared in triplicate.

2.2. Particle size determination

Particle size and particle size distribution function were analysed by the dynamic light scattering technique on a Zeta Plus instrument (Brookhaven Instruments, USA) (measurement parameters were set as follows: refractive index of particles 1.400, refractive index of solvent (isopropanol) 1.3770, solvent dynamic viscosity 2.04 cP (2.04 mPa s), measurement angle of 90°, incident light wavelength of 658 nm). An approximate 0.5 g sample of chocolate was diluted in 10 ml of isopropanol (Sigma Aldrich, USA) and subsequently heated and sonicated at 55 °C temperature for 60 min (sonic bath power set on 10% at 35 kHz frequency) (Ultrasonic bath type K2L (Kraintek, Czech Republic). Afterwards, prior to each measurement, diluted chocolate samples were vigorously hand shaken (Saputro et al., 2017).

2.3. Hardness determination

The TA.XT plus texture analyser (Stable Micro Systems, UK) was used for hardness measurements of the prepared milk chocolate samples. The measurements were set as the single penetration event. Sample hardness analysis was performed at the temperature of (22 ± 1)°C. Each sample was analysed a minimum of nine replicates. The obtained results were expressed as the mean value and the corresponding standard deviation. Measurement parameters were identical with those given in literature (Lillah, Asghar, Pasha, Murtaza, & Ali, 2017). The hardness of the samples was determined by penetrating an aluminium probe into the tested sample. A probe diameter was of 2 mm (P/2N) and penetration rate of 2 mm/s was used. Penetration depth was set to 5 mm.

2.4. Thermal analysis of chocolate samples

The thermal behaviour of the chocolate samples was characterised by differential scanning calorimetry measurements (DSC, Mettler Toledo, Switzerland). The apparatus was calibrated using indium as the standard. Approximately (10.0 ± 0.5) mg of the studied samples were grated and placed into aluminium pans. The pans were then hermetically sealed to maintain constant moisture and air atmosphere

Table 1
Studied samples labelling and composition.

Sample labelling	Composition (w.%)					
	Cocoa mass	Cocoa butter	Saccharose	Lecithin	Skimmed milk powder	Whey powder
Control sample	13.0	27.5	33.0	0.5	26.0	0
CH_w_2_5	13.0	27.5	33.0	0.5	23.5	2.5
CH_w_5	13.0	27.5	33.0	0.5	21.0	5.0
CH_w_7_5	13.0	27.5	33.0	0.5	18.5	7.5
CH_w_10	13.0	27.5	33.0	0.5	16.0	10.0

conditions during measurements. An empty pan was used as the reference. During DSC measurements of the studied samples, the following thermal cycles were applied: temperature was kept constant at 15 °C for 5 min followed by linear heating from 15 to 250 °C with the 10 °C/min heating rate, then followed by cooling from 250 to 15 °C (with the 10 °C/min cooling rate), kept constant at 15 °C for 5 min, heated from 15 to 250 °C (with the 10 °C/min heating rate), and finally cooled from 250 to 25 °C (with the 50 °C/min cooling rate). All measurements were performed in a nitrogen atmosphere. The applied nitrogen flow rate was 50 ml/min (Agibert & Lannes, 2018). DSC thermograms were characterised by the detection of the following specific temperatures points: T_o (onset), T_p (peak), and T_e (endset) temperatures (Ziegleder, 1990), as well as of the T_{cp} – sucrose melting peak temperature; $T_{\alpha mp}$ – α-lactose monohydrate melting peak temperature. Fusion enthalpies associated with the structural changes in whey chocolate matrices, e.g., cocoa fat polymorphism, whey lactose crystallisation, sucrose melting, etc., were determined from the calculated area under the obtained DSC curves and expressed in normalised values of ΔH (J/g). The melting profile and the moisture content of chocolate samples were measured on simultaneous DSC/TGA, SDT 650 Discovery with TRIOS software for thermal analysis (TA Instrument, USA). The apparatus was calibrated using indium as a standard. Prior to each measurement, samples were grated into an aluminium pan. The samples weight was approx. (15 ± 1) mg. Open pans were used for the measurements. The following experimental conditions were set: equilibrium at 25 °C, heating to 150 °C, applied heat rate of 5 °C/min. Experiments were performed in a nitrogen atmosphere. The nitrogen flow rate was of 100 ml/min. The results of this analysis were expressed as T_o – onset temperature of chocolate melting; T_{mp} – chocolate melting peak temperature; T_e – endset temperature of chocolate melting; T_d – whey protein denaturation peak temperature.

2.5. Rheological measurements

The rheological properties of studied samples were determined on a HAAKE RheoStress 1 oscillation rheometer (Thermo Fisher, USA). Particular analysis of the studied samples was performed in the following time scales: one day, one week and two weeks after their preparation. Each measurement was performed in triplicate. The plastic viscosity and the yield strength (shear stress) of the tested samples were determined. Plate to plate sensor geometry was used (sensor diameter was 30 mm, measurement temperature (40.0 ± 0.5)°C). Measurements were performed according to the methodology described by Glicerina et al. (Glicerina, Balestra, Dalla Rosa, & Romani, 2016). The obtained flow curves were fit to the Casson's model in order to determine plastic viscosity and yield strength. Thixotropy values were also evaluated (Glicerina et al., 2016).

2.6. Acoustic measurement

Acoustic emissions generated upon breakage of the studied chocolate samples were evaluated by the detection of the sound pressure level L_p (dB) in the course of the manual chocolate bars fracture. L_p is defined as follows (Kim, Jang, & Kim, 2010; Sprague & Luczkovich, 2004):

$$L_p = 20 \cdot log \frac{p}{p_0}, \quad (1)$$

where: p (Pa) is the root mean square sound pressure and p_0 (Pa) is the reference sound pressure (20 μPa in the air atmosphere). The A-weighted peak sound pressure levels L_{pAmax} (dB) (Svec & Granqvist, 2018) were determined by use of the Voltcraft sound level meter SL-400 (Conrad Electronic SE, Hirschau, Germany). All measurements were performed in the acoustic chamber. Samples were broken at a perpendicular distance of 50 mm from the microphone of the sound level meter. Each type of chocolate was measured 5x at the laboratory temperature of (22 ± 1)°C.

2.7. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to visualise the microstructure of the chocolate bars' fracture surface (Afoakwa et al., 2009). The lyophilisation process was employed to remove moisture content from the chocolate samples. The freeze-dried samples were sputter coated with gold-palladium to make them electrically conductive. SEM images were captured using a Hitachi 6600 FEG microscope (Japan) operating in the secondary electron mode using an accelerating voltage of 1 keV (Lapcik et al., 2020).

2.8. Statistical analysis

Data was analysed using one-way analysis of variance (ANOVA method). Differences in the mean values among statistical groups were tested at a 0.05 significance level. SigmaStat version 2.03 statistical software (Systat Software, Inc., USA) was used for data analysis. All experiments were performed on chocolate samples prepared in at least three replicates.

3. Results and discussion

3.1. Particle size analysis

The results of particle size analysis of diluted dispersions of the studied milk chocolate samples are shown in Fig. 1. The obtained dependencies exhibited a decreasing trend of the observed particle mean diameter with increasing whey content ranging from 75.3 nm (control sample) to 34.7 nm (10 w.% whey content) chocolate dispersions measured at 25 °C without sonication (Bylund, 1995; Saputro et al., 2017). We also observed similar trends for samples prepared at 40 °C for both the sonicated samples as well as samples without sonication. The median diameter d_{50} also decreased by a factor of approximately one half compared to the control sample. Fortification of the chocolate samples with whey lead to the narrowing trend of the cumulative undersize distributions (d_{90}-d_{10}) as given in Table 2. The latter indicated narrowing of the particle sizes was the smallest for the sonicated dispersions. The polydispersity index was found to be 0.005, which corresponds to monodisperse systems. It means that the observed particle sizes of the mean diameter were from the specific narrow range of magnitudes. Previous literature (Coutinho et al., 2019) determined that small particles of the dairy component of a chocolate drink lead to increased system dispersability. This effect was due to the occupation of the free spatial volume present between large particles by small ones, thus resulting in the occurrence of the lubrication effect, hence the

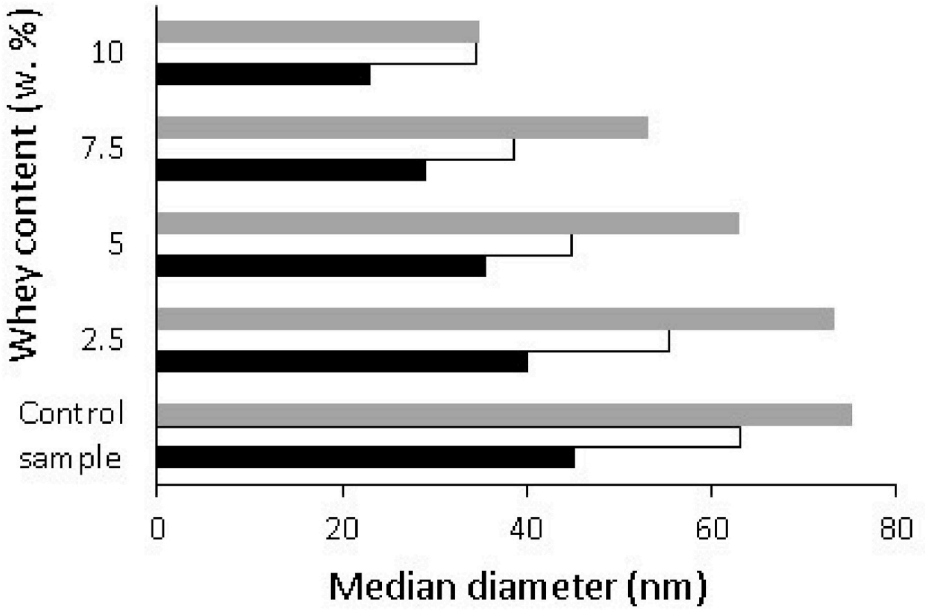

Fig. 1. Observed whey content dependencies of the median particle diameter of milk chocolate dispersions prepared at different conditions: grey column – at the temperature of 25 °C, white column – at the temperature of 40 °C, black column – sonicated at the temperature of 40 °C.

Table 2
Observed mean diameters of dispersions of studied chocolate samples.

Sample (w.% whey)	Mean diameter (nm)	d_{10} (nm)	d_{50} (nm)	d_{90} (nm)
At 25 °C without sonication				
Control sample	75.3 ± 0.9 [a]	68.7 ± 1.4 [a]	75.2 ± 0.6 [a]	82.3 ± 1.0 [a]
CH_w_2_5	73.4 ± 0.8 [a]	66.8 ± 0.9 [a]	73.2 ± 1.3 [a]	80.1 ± 0.9 [a]
CH_w_5	63.1 ± 1.1 [b]	57.5 ± 1.2 [b]	63.0 ± 1.1 [b]	68.9 ± 1.2 [b]
CH_w_7_5	53.4 ± 0.7 [c]	48.6 ± 0.8 [c]	53.2 ± 1.4 [c]	58.3 ± 0.6 [c]
CH_w_10	34.7 ± 1.2 [d]	31.7 ± 1.0 [d]	34.7 ± 0.7 [d]	37.9 ± 0.8 [d]
At 40 °C without sonication				
Control sample	63.3 ± 0.5 [a]	57.7 ± 0.8 [a]	63.1 ± 0.9 [a]	69.1 ± 0.6 [a]
CH_w_2_5	55.5 ± 0.9 [b]	50.4 ± 1.0 [b]	55.3 ± 0.5 [b]	60.6 ± 0.8 [b]
CH_w_5	45.0 ± 1.1 [c]	41.0 ± 1.1 [c]	44.9 ± 1.2 [c]	49.1 ± 1.0 [c]
CH_w_7_5	38.7 ± 0.7 [d]	35.3 ± 0.8 [d]	38.6 ± 1.1 [d]	42.3 ± 0.7 [d]
CH_w_10	34.9 ± 0.6 [e]	31.8 ± 0.9 [e]	34.8 ± 0.6 [e]	38.1 ± 1.0 [e]
At 40 °C with sonication				
Control sample	45.2 ± 0.7 [a]	40.1 ± 0.7 [a]	45.1 ± 1.1 [a]	51.6 ± 0.8 [a]
CH_w_2_5	40.3 ± 0.8 [b]	36.7 ± 0.9 [b]	40.2 ± 0.6 [b]	44.0 ± 1.1 [b]
CH_w_5	49.8 ± 0.9 [c]	45.4 ± 1.2 [c]	49.7 ± 0.8 [c]	54.4 ± 0.8 [c]
CH_w_7_5	29.2 ± 0.9 [d]	26.6 ± 0.5 [d]	29.1 ± 1.0 [d]	31.9 ± 0.9 [d]
CH_w_10	23.2 ± 0.8 [e]	21.2 ± 0.7 [e]	23.2 ± 0.9 [e]	25.4 ± 1.3 [e]

Legend: c. s. – control sample; d_{10} – maximal diameter representing 10% of whey chocolate particles share; d_{50} – maximal diameter representing 50% of whey chocolate particles share; d_{90} – maximal diameter representing 90% of whey chocolate particles share. Values are the mean ± standard deviation of three measurements. Superscripts with different letters in the same column indicate significant differences between the samples ($\alpha \leq 0.05$); data with the same letters in the same column are not significantly different as determined using Tukey test.

observed decrease of system viscosity (Afoakwa et al., 2008; Saputro et al., 2017). Later findings were in excellent agreement with our textural and rheological measurements given in Table 3 and Section 3.2.

3.2. Rheological measurements

The calculated parameters of Casson's rheological model of experimental flow curves of the studied chocolate melts are given in Table 3. Rheological flow curves of chocolates are characteristic with pseudoplastic rheological patterns exhibiting characteristic yield stresses. Many factors affect the later rheological behaviour such as the applied production technological process (grinding, conching, tempering, storing temperature) and the chocolate formulation compositions (i.e. amount of cocoa butter, amount and type of used emulsifier, dispersed particle size, moisture content, etc.) (Fernandes, Mueller, & Sandoval, 2013; Glicerina, Balestra, Rosa, & Romani, 2015). The rheological properties of chocolate are known to also be strongly influenced by the addition of dairy ingredients into the final chocolate composition. The application of those with a higher amount of free milk fat reduce the viscosity of chocolate. However, the material properties of the applied dairy ingredients themselves, i.e., surface properties, particle size and method of extraction also seem to be decisive (Lapcik et al., 2015; Lapčík et al., 2017). Decreasing Caisson's plastic viscosity with increasing whey

Table 3
Calculated textural and rheological properties of studied milk chocolate melts with varying whey concentrations.

Whey concentration (% w/w)					
Parameter	Control sample	2.5	5.0	7.5	10.0
[a]Hardness (N)	73.3 ± 2.3	56.8 ± 2.2	55.1 ± 2.1	53.1 ± 1.5	52.1 ± 3.5
[b]Casson yield stress (Pa)	3.8 ± 0.09	3.66 ± 0.05	3.54 ± 0.04	3.49 ± 0.06	3.23 ± 0.07
[b]Casson plastic viscosity (Pa.s)	1.47 ± 0.07	1.42 ± 0.08	1.41 ± 0.06	1.39 ± 0.05	1.34 ± 0.03
[b]Thixotropy (Pa)	80.0 ± 1.6	64.0 ± 1.1	48.1 ± 2.6	48.0 ± 1.6	32.0 ± 1.4

[a] Measured at (22 ± 1) °C.
[b] Measured at (40.0 ± 0.5) °C.

concentration, as given in Table 3, was also confirmed in this study. However, the observed differences between the individual samples were not significant in this study. The addition of whey powder generally had a viscosity thinning effect on the model milk chocolate matrices. Nevertheless, model samples without whey powder addition displayed the highest apparent viscosity values, while the lowest values were observed for samples with 10 w.% whey powder concentration. We assumed that the observed decrease of the viscosity with increasing addition of whey powder was due to the increase of the amount of free milk fat in the chocolate mixture. Another possible explanation may be that this is due to changes of the complex microstructure of the bulk chocolate matrix existing between all solid components, thus influencing cocoa and cocoa fat crystallisation (Glicerina et al., 2016; Beckett, S. T., Fowler, & Ziegler, 2017). The observed yield stress value data ranged from 3.23 to 3.80 Pa. In particular, samples without whey component were of the highest yield stress magnitudes, while samples with the addition of 10 w.% whey powder exhibited the lowest yield stress values. The latter trend was similarly seen in the viscosity patterns attributed to the change of the amount of free milk fat present in the chocolate mixture (Glicerina et al., 2016; De Graef, Depypere, Minnaert, & Dewettinck, 2011).

Thixotropy is a rheological property exhibited by many pseudoplastic food systems. When the chocolate is produced and left to "stand", a three-dimensional internal structure is formed. However, whereas if the chocolate is again exposed to shear friction deformation, a newly formed three-dimensional arrangement disintegrates. The thixotropy magnitude of the studied milk chocolate model samples ranged from 32.0 to 112.0 Pa. Whey free samples, or those with the lowest addition of whey component, exhibited the highest thixotropy of about 112.0 Pa. For the higher whey concentrations, a decreasing thixotropy trend was found indicating better matrix homogenisation. Similarly, as mentioned earlier in this section, this trend was attributed to the increased amount of free milk fat in the system, thus reducing the number of interactions between solid components and hence affecting the microstructure of the bulk matrix (Glicerina et al., 2016; Beckett et al., 2017; Glicerina et al., 2015).

3.3. Hardness

In general, hardness is an important property of chocolate because it is very closely related to its sensory quality (Hrivna, Machalkova, Buresova, Nedomova, & Gregor, 2021). The hardness of chocolate is the result of interactions between the crystallised continuous fat phase and the dispersed solid particles. The hardness of chocolate is influenced by the production process, the properties of the raw materials used (size, shape, surface), but also by the length and storage conditions. Moreover, the hardness of milk chocolate is generally higher in comparison with white chocolates, and lower compared to dark chocolates. The hardness decreases with the addition of the milk component due to its milk fat content, which is proportionally changing the content of fatty acids in the continuous phase. Milk fat softens chocolate, lowers its melting point and thus facilitates its melting properties in the mouth (Glicerina et al., 2016; Attaie, Breitschuh, Braun, & Windhab, 2003; Chire-Fajardo, Ureña-Peralta, & Hartel, 2020; Daza-La Plata, Chire-Fajardo, & Ureña-Peralta, 2020; Ostrowska-Ligęza et al., 2019; Machálková, Hrivna, Nedomová, & Juzl, 2015). The milk chocolate sample hardness values ranged from 52.1 to 73.3 N, of which the lowest hardness values were observed for samples with 10 w.% addition of whey powder (52.1 ± 3.5) N and, conversely, the highest values were presented in samples without the addition of whey powder after two weeks of storage (73.3 ± 2.3) N. In general, the softening effect of additional whey powder on the milk chocolate sample models was observed. These results were in agreement with previous study (Pandey & Singh, 2011). However, the obtained hardness values were lower in comparison to that reported in the above-mentioned study (Pandey & Singh, 2011). The reason for this effect may be due to the higher content of milk components present in the composition, or due to the different application of processing parameters. In addition, the higher storage temperatures could also result in a decrease of the chocolate hardness. The effect of storage temperature on chocolate quality has been described elsewhere (Ruban, Hrivna, Machálková, Nedomova, & Sottnikova, 2016). The latter authors tested the hardness of milk chocolate (cocoa components 35 w.%) at different storage temperatures. In particular, the hardness was also tested at the temperature of 6 °C, which also corresponds to the storage temperature applied in our study. Ruban et al. (Ruban et al., 2016) reported hardness values of about 46.0 N immediately after production. After one month of storage, the tested chocolate reported a hardness of 60.0 N. Furthermore, magnitudes of the hardness were similar to that of the model sample with 10 w.% addition of whey powder (52.1 ± 3.5) N. On the other hand, higher hardness values of (96.7 ± 10.5) N were reported for milk chocolate with 25 w.% cocoa components (Ostrowska-Ligęza et al., 2019). Moreover, a practical benefit of this study could be consideration that for milk chocolate, in which softer consistency is desired, the application of whey powder could be recommended. On the contrary, for milk chocolate in which a firmer mouth feel is required, the use of whey powder should be limited.

3.4. Thermal analysis

The strong effect of the applied mass ratios of the cocoa butter and the lactose added in the form of whey or skimmed milk powders on the prepared chocolates thermal behaviour was confirmed in this study. A DSC technique was also used for characterization of chocolate samples with different whey powder content in other literature (Afoakwa et al., 2008). Chocolate samples represent complex systems characterised by the occurrence of multiple peaks during heating and cooling cycles. The first endothermic peak on the DSC curve after cooling to 15 °C was detected at a temperature of (19.67 ± 0.17)°C with the corresponding fusion enthalpy (ΔH) of (20.68 ± 1.00) J/g. This peak was associated with the melting of α − cocoa butter of the polymorphic form present in the chocolate. It was the same for all samples under this study, independent of the applied whey content. This result was in agreement with previously published studies (Ostrowska-Ligęza et al., 2019; Barišić et al., 2019; Pirouzian et al., 2020). The melting profiles of studied chocolate samples in the temperature range close to the cocoa butter melting point by means of DTA analysis were determined there. Open pans were used for the measurements. As can be seen from Fig. 2, with increasing whey content, the melting point peak was shifted to lower temperatures accompanied with a minor decrease of the magnitude of the fusion enthalpy as given in Table 4. The addition of the whey component into the chocolate matrix provides an increase of its hydrophilic character (Lapčík et al., 2017). The rate of the formal melting kinetics of the cocoa butter was calculated from the tangent in the inflection point of the observed heating curves in the temperature range from 30 to 31 °C as shown in Fig. 2. The calculated steepness magnitudes increased with the increasing whey content of the samples indicating an increasing rate of the melting process. We expected that the whey particles present in the chocolate matrix slightly hinders the process of cocoa butter crystallisation in such a way that the whey particles are embedded into the chocolate matrix as evident from the SEM images shown in Fig. 5, thereby forbidding the creation of large crystalline bulk regions. The whey protein denaturation peak was observed in the temperature range from 86.3 to 85.97 °C. It was evaluated from the 40–150 °C temperature range. Observed fusion enthalpies differed in their magnitudes, ranging from 265.72 to 416.7 J/g corresponding with the increasing whey content as given in Table 3. Observed thermal events were related to the creation of protein lipid complexes in the chocolate structure (Wang et al., 2020). The control sample exhibited a typical crystallisation peak at the temperature of 213.6 °C. The shifting of the latter fusion temperature to the higher temperatures with an increasing milk lactose content were found there as well. This finding was in excellent agreement with previous findings in literature (Konar

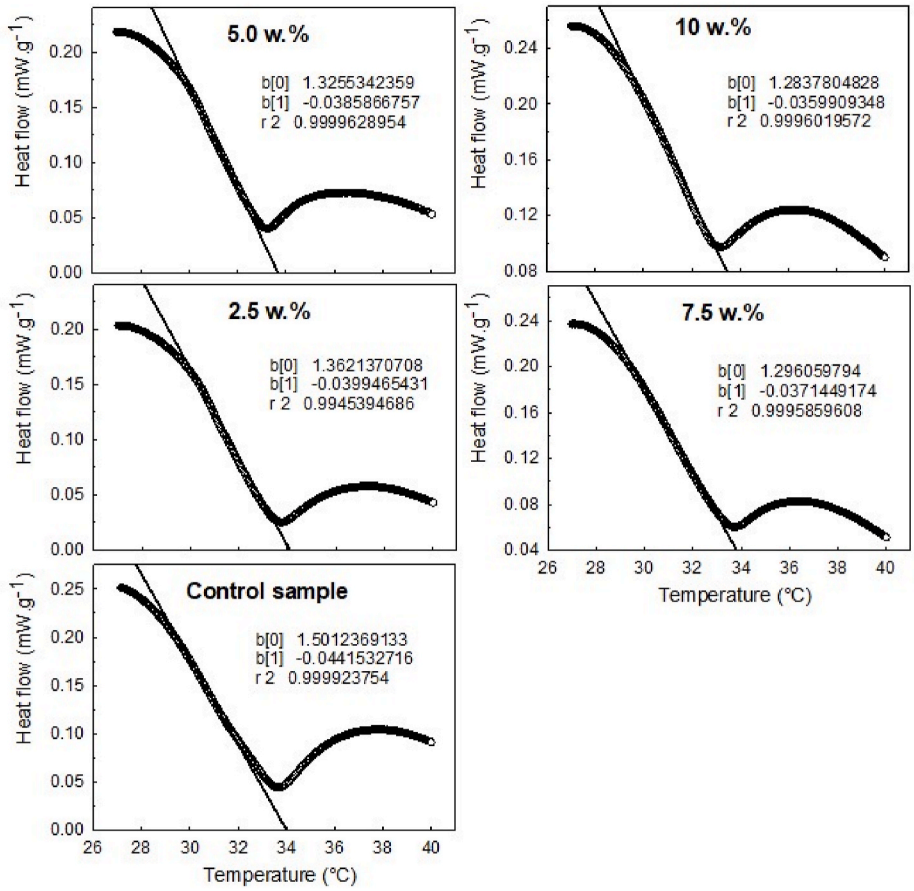

Fig. 2. DSC patterns of the melting peak temperatures with tangent line in the inflection point of chocolate samples of different whey content.

Table 4
Melting properties of studied chocolate samples of varying whey content.

Sample (w.% whey)	T_o (°C)	T_{mp} (°C)	T_e (°C)	ΔH_m (J/g)	T_d (°C)	ΔH_d (J/g)	T_{cp} (°C)	ΔH_c (J/g)	T_{amp} (°C)	ΔH_{am} (J/g)
Control sample	28.13 ± 0.11 ^{a}	34.10 ± 0.12 ^{a}	38.61 ± 0.11 ^{a}	19.91 ± 0.26 ^{a}	–	–	157.99 ± 0.11 ^{a}	50.03 ± 0.12 ^{a}	213.60 ± 0.10 ^{a}	79.40 ± 0.21 ^{a}
CH_w_2_5	30.07 ± 0.08 ^{b}	33.52 ± 0.06 ^{bc}	35.56 ± 0.05 ^{b}	3.90 ± 0.17 ^{b}	86.30 ± 0.09 ^{a}	265.72 ± 0.21 ^{a}	158.60 ± 0.12 ^{b}	51.51 ± 0.10 ^{b}	200.05 ± 0.14 ^{b}	114.17 ± 0.15 ^{b}
CH_w_5	29.67 ± 0.05 ^{c}	33.71 ± 0.10 ^{b}	35.33 ± 0.04 ^{c}	9.85 ± 0.23 ^{c}	86.04 ± 0.03 ^{b}	301.63 ± 0.19 ^{b}	158.01 ± 0.14 ^{a}	49.59 ± 0.13 ^{c}	197.78 ± 0.07 ^{c}	136.46 ± 0.07 ^{c}
CH_w_7_5	29.47 ± 0.07 ^{c}	33.38 ± 0.08 ^{c}	35.17 ± 0.05 ^{c}	2.87 ± 0.14 ^{d}	86.07 ± 0.02 ^{b}	369.60 ± 0.15 ^{c}	159.88 ± 0.10 ^{c}	44.62 ± 0.09 ^{d}	198.50 ± 0.05 ^{d}	147.80 ± 0.10 ^{d}
CH_w_10	29.58 ± 0.08 ^{c}	32.93 ± 0.10 ^{d}	34.85 ± 0.10 ^{d}	2.90 ± 0.12 ^{d}	85.97 ± 0.04 ^{b}	416.70 ± 0.18 ^{d}	159.05 ± 0.16 ^{d}	37.31 ± 0.10 ^{e}	198.24 ± 0.11 ^{d}	141.42 ± 0.12 ^{e}

Legend: c. s. – control sample; ΔH – enthalpy change of relevant thermal event; T_o – onset temperature of chocolate melting; T_{mp} – chocolate melting peak temperature; T_e – endset temperature of chocolate melting; T_d – whey proteins denaturation peak temperature; T_{cp} – sucrose melting peak temperature; T_{amp} – α-lactose monohydrate melting peak temperature.
Values are the mean ± standard deviation of three measurements. The hyphen means that no value was detected. Superscripts with different letters in the same column indicate significant differences between the samples ($\alpha \leq 0.05$); data with the same letters in the same column are not significantly different as determined using Tukey test.

et al., 2017).

Parameters of the second endothermic peak (DSC) are shown in Table 3. The latter peak was observed in the temperature range from 157.99 to 159.05 °C (based on DSC measurements). The appearance of this peak was ascribed to the corresponding melting of the sugar phase present in the chocolate (Beckett, Stephen T., Francesconi, Geary, Mackenzie, & Maulny, 2006). With increasing whey content, the magnitudes of the latter enthalpies decreased when accompanied by their shift to higher temperatures. This phenomenon was ascribed to the plasticising effect of the whey and lecithin present in the studied chocolate matrix. The sucrose content of 33 w.% was kept constant for all tested recipes, and this component represented the solids matter dispersed in the fat acting as the dispersing medium (Aaltonen et al., 2020). The obtained results corresponded with earlier observations found for chocolates modified with sucrose – palm sugar blends (Saputro et al., 2017). As shown in Fig. 3, the endothermic peak was shifted to a lower temperature from 200.0 to 198.2 °C with increasing whey content; an obviously higher peak temperature of 213.6 °C, due to the lactose crystallisation of the skimmed milk powder was detected for the control sample (without any addition of whey) (Lapcik et al., 2015). We

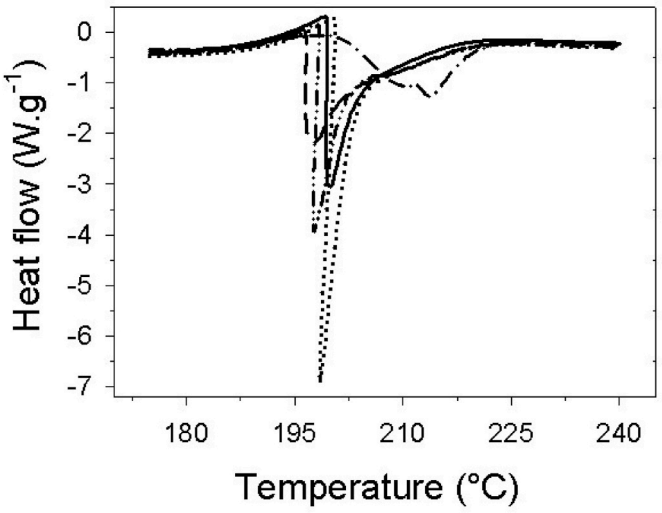

Fig. 3. DSC patterns of chocolate samples in the temperature range from 175 to 240 °C. Whey content: Dash–dot line – control sample, solid line – 2.5 w.%, long dash - 5 w.%, dotted line – 7.5 w.%, dash-dot-dot line – 10 w.%.

assume that the temperature peak detected for samples with whey powder represents an α-lactose monohydrate melting point, which was detected at 202 °C for pure lactose (Thomas, Scher, & Desobry, 2004). This peak might be attributed to water released from the collapsed whey lactose structure. The magnitudes of the corresponding fusion enthalpies increased with increasing whey content, as given in Table 4, similarly to this observation.

Decreased water release with increasing whey content from 2.2 w.% (control sample) to 0.9 w.% (10 w.% whey content), suggesting the ability of whey to retain water in its structure, were determined from TGA measurements in the temperature range of 25–150 °C (Lillah et al., 2017).

3.5. Acoustic measurements

The sound emission upon breakage of chocolate is an important perception parameter of some chocolate products together with other textural and sensory parameters (Badak-Kerti, Zsom-Muha, Zsom, Nagy, & Felföldi, 2020). Due to the fact that the main chocolate and chocolate-based product constituents are mixtures of cocoa fat and other vegetable oils, the mechanical properties of the latter fat matrices are strongly affected by their crystalline fat content. Other factors are crystal size, morphology, degree of crystal aggregation and presence of solid bridges (Gregersen et al., 2016). As the crystallisation of fat occurs when liquid fat is cooled below melting point and the resulting supersaturation allows growth of the crystallisation nuclei (Gregersen et al., 2016), the thermal history of the chocolate production and storage is an important quality parameter (Hrivna et al., 2021). Chocolate breakage creates acoustic emissions as the consequence of stress waves generated in the matrix. This triggers stress induced energy accumulation in the material followed by its subsequent rapid release as the microstructural changes proceed. The results of acoustic emissions measurement during chocolate samples breakage are shown in Fig. 4. It was found that the observed acoustic emissions (peaks sound pressure levels L_{pAmax}) during breakage decreased with increasing whey content. L_{pAmax} decreased from 61.9 dB to 54.3 dB (of 2.5 w.% and 10 w.% whey content). This indicated the change of the fracture mechanism from brittle to ductile. These results were in an excellent agreement with the hardness measurements, where the hardness was decreased from 73 N, as observed for the control sample, to 52 N, as observed for the 10 w.% whey sample. This indicated an ongoing plasticising effect of the whey spherical particles on the chocolate matrix via the hindrance of creation of wide crystalline fat aggregates regions as discussed further in the next SEM Section.7.

The latter fact was also confirmed by the decreased A-weighted peak sound pressure levels L_{pAmax} from 61.9 dB to 54.3 dB (of 2.5 w.% and 10 w.% whey content) during chocolate breakage. Decreased acoustic emissions thus indicated the fracture process change from brittle to more ductile.

3.6. Scanning electron microscopy (SEM)

Results of the SEM analysis of the tested original and whey-modified milk chocolates are shown in Fig. 5. Typical cocoa fat crystallite patterns (Teodoro da Silva, Grimaldi, Calligaris, Cardoso, & Guaraldo Goncalves, 2017) are distinguished here together with the characteristic spherical whey particle shape (Lapcik et al., 2015). A visible trend of decreasing crystallite ordered structure, typical for the control sample milk chocolate, to the more amorphous-like (or so called ill-defined crystal structures (Afoakwa et al., 2008)) as shown in Fig. 5 for 2.5 w.% whey concentration. The literature confirmed that the crystallite structure order is maintained for the cocoa butter down to the nanoscale dimensions of the individual crystals (Jacobs & Barroo, 2021). Such molecular ordering might be responsible for the creation of highly dense crystallites formed from the individual nano-sized crystals.

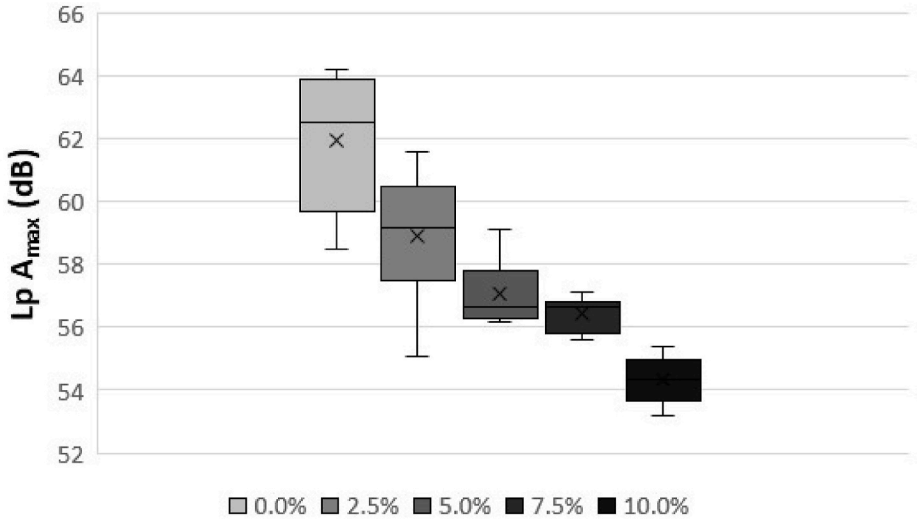

Fig. 4. Acoustic properties of studied milk chocolates of varying whey content (w.%).

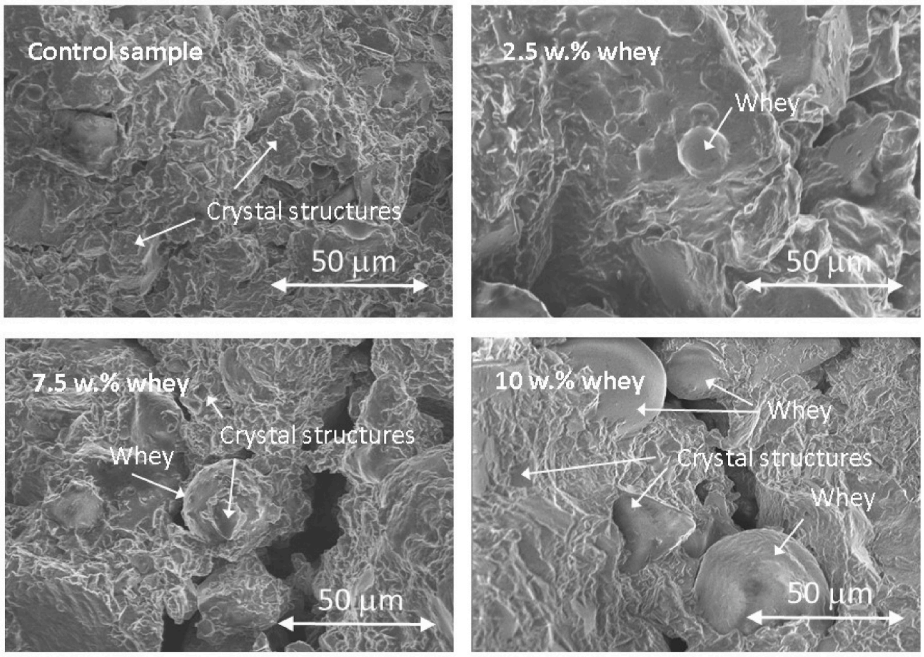

Fig. 5. SEM images of the studied whey chocolate samples.

4. Conclusion

This study determined that the complex microstructure of chocolate is modified by the addition of whey into original milk chocolate in such a way that the higher ordered dense crystalline cocoa fat matrix is weakened by the inclusion of the spherical whey particles, thus disrupting the higher order crystalline structure of the cocoa fat aggregates. Dynamic light scattering experiments further determined a decreasing cocoa fat particle size diameter for non-sonicated dispersions from 75.3 nm to 34.1 nm for whey contents of 2.5 w.% and 10 w.%, respectively. Observed crystallinity changes were ascribed to the modification of highly ordered dense cocoa fat crystal aggregates structures with the inclusion of whey particles, as confirmed by SEM analysis. These changes affected the thermal and rheological behaviour of the studied samples by decreasing melting peak temperature from the original 33.52 °C–32.93 °C for whey modified chocolate, as well as Casson's plastic viscosity from 1.42 Pa s to 1.34 Pa s and thixotropy from 64 Pa to 32 Pa for increased whey content from 2.5 w.% to 10 w.%. Furthermore, the gradual decrease of the hardness, thus reflecting the plasticising effect of the whey particles on the complex crystalline structure of the chocolate was also observed there. The latter fact was additionally confirmed by the decreased A-weighted peak sound pressure levels L_{pAmax} from 61.9 dB to 54.3 dB (2.5 w.% and 10 w.% whey content) during chocolate breakage. Decreased acoustic emissions thus indicated the fracture process change from brittle to more ductile.

CRediT authorship contribution statement

Barbora Lapčíková: Funding acquisition, Investigation, Methodology, Data curation, Formal analysis, Writing – original draft. **Lubomír Lapčík:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Richardos Salek:** Samples preparation, Investigation, Data curation, Formal analysis, Writing – original draft. **Tomáš Valenta:** Investigation, Software, Data curation, Formal analysis, Writing – original draft. **Eva Lorencová:** Investigation. **Martin Vašina:** Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Financial support from the internal grants of Palacky University in Olomouc (project number IGA_PrF_2021_031) and of Tomas Bata University in Zlin (project number IGA/FT/2021/004) are gratefully acknowledged. Authors would like to express their special thanks to Dr. K. Čépe, Ph.D. (CATRIN, Palacky University in Olomouc) for SEM measurements.

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