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CHARACTERIZATION OF THERMAL PROPERTIES OF FATS EXTRACTED FROM MILK, WHITE, DARK CHOCOLATES AND CHOCOLATE POWDER BY DIFFERENTIAL SCANNING CALORIMETRY (DSC) TECHNIQUE

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Abstract. The effect of different composition of dark, milk, white chocolate and chocolate powder samples was investigated on their thermal characteristics. Thermal characteristic was estimated by means of differential scanning calorimetry (DSC). The research is covered to determine melting characteristics of fats extracted from 36 dark (D1 - D36), 23 milk (M1 - M23), 8 white (W1–W8) chocolates samples, as well as, 2 dark and 2 milk chocolate powder (DP1 - DP2, MP1 - MP2) samples.

The results obtained of the melting point of extracted fats are related to their physical properties, such as thermal behavior. There are described the utilization of DSC technique on the determination of melting points of extracted fats from different species of chocolate, including chocolate powder.

According to the melting characteristics of fats extracted from chocolate species and chocolate powder, it was studied that in DSC curves have observed similarities and differences due to the melting points.

Keywords: *chocolate samples, chocolate powder, extracted fats, DSC.*

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1. Introduction

There are different types of chocolates (dark, milk and white), according to their composition in terms of cocoa solids, cocoa butter and milk fat, hence the final products contain different compositions of carbohydrate, fat and protein (Beckett, 2008; Fernandes *et al.*, 2013). Chocolate processing is related to the crystallization and polymorphic of the fat phase and explained by tempering chocolate which is necessary to crystallize the cocoa butter in a stable polymorphic form (Beckett, 2009). While Wille and Lutton (1966) were using Form I and Form VI to define the polymorphism of cocoa butter, Larsson (1966) used this Greek letter nomenclature and the stability, as well as the melting point of the polymorphic form increased from γ to α, to β'', to β (Langevelde *et al.*,1999; Larsson, 1966; Wille & Lutton, 1966). Milk fat is considered polymorphic due to its structure and may crystallize taking three different polymorphic forms: γ, α, and β′. The most stable form of the milk fat is a form of β′ and the least stable form γ (Grotenhuis *et al.*, 1999; Lopez *et al.*, 2001; Wright *et al.*, 2000).

Thermal analysis is recognized as an instrumental method of food analysis that is able to give unique information regarding the nature of the sample or the modifications induced by industrial processing. Books and reviews report the applications of thermal-analytical techniques to the food science (Gabbot, 2007; Kemp, 1999; Materazzi *et al.*, 2017; Tieko & Gonçalves, 1999). Thermal analysis, such as differential scanning calorimetry (DSC) can be used in isotherm or non-isotherm mode, by providing information about melting characterization, crystallization, oxidative stability and fat content of different mixture, as well as thermogravimetric analysis (TGA) can be used to determine the composition of dark and milk chocolate and consequently to control the quality of product (Materazzi *et al.*, 2014; Ostrowska-Ligęza *et al.*, 2018; Pardauil *et al.*, 2017). During chocolate manufacture, the melting character in the final product depends on the crystalline state and the proportion of existing solid fat (Afoakwa *et al.*, 2008). Melting profiles of chocolates are expected to differ due to various fatty acids composition and changeable amount of fine solid particles (sugar and cocoa) based on type of chocolate (Dolatowska-Zebrowska *et al.*, 2019).

In conduct to the polymorphism, tempering is important in order to provide crystallization in a thermodynamically stable polymorphic form. In this case, tempering reduce temperature of chocolate to compel both stable and unstable polymorphs (Beckett, 2009). Studies by Ostrowska-Ligęza *et al.*, (2019) determined the melting characteristics for cocoa butter and milk fat before addition to chocolate and after extraction from chocolate were very similar. Furthermore, Dolatowska-Zebrowska *et al.*, (2019) analyzed that the fat extracted from goat's milk chocolate was different from goat milk fat according to their DSC melting curves.

The aim of this study was to determine the melting characteristics of fats extracted from dark, milk, white chocolate and chocolate powder on DSC. It led to assess the range of temperature of the polymorphic forms of fats into different chocolate species. Moreover, determination of the temperature ranges of the polymorphic forms of fats extracted from chocolate powder was on the object of the study.

2. Materials and method

2.1. Materials

The research material consisted of 36 dark (D1 - D36), 23 milk (M1 - M23) and 8 white chocolates samples (W1 – W8), which were purchased at a local store in Poland. Additionally, milk and dark chocolate powder samples were provided by Department of Chemistry of Warsaw University of Life Sciences.

2.2. Extraction of lipid fraction

Chocolate samples were ground separately before extraction. Fats were extracted according to the procedure described by Boselli *et al.* (2001). Approximately, 10 g minced sample of all the chocolates (included chocolate powder samples) were homogenized severally with 100 mL of a chloroform/methanol solution (1/1 v/v) in a glass bottle with a screw-cap. The bottle was kept at 60 °C for 20 min before adding 100 mL of chloroform. After 3 min of homogenization, the solution was filtered to get rid of unresolved pieces of chocolate and washed with chloroform. A filtrate was mixed thoroughly with 70 mL of 1 N KCL solution and left overnight at 4 °C in order to phase separation. The organic phase was collected, added 2-3 spoonful of anhydrous magnesium sulfate and kept in a dark place for 2 hours. In order to separate the solvent from the filtrate fat they were evaporated by the rotary evaporator at 40 °C within initial pressure of ~422 m bar and final pressure of ~100 m bar, and dried at nitrogen N_2 (Folch *et al.*, 1957; Wirkowska *et al.*, 2012). The fats which extracted from dark, milk, white chocolate samples and chocolates powder samples were stored at − 20 °C until they were analyzed.

2.3. Melting characteristics of fats extracted from milk, dark, white chocolate samples and chocolate powder samples

DSC measurements of melting characteristics were carried out with a Q200 DSC (TA Instruments, New Castle, DE, USA). Calibration was done with indium standards. For extracted fats, approximately 3–4 mg was placed into aluminum pans with a lid and was hermetically sealed. An empty sealed aluminum pan was used as a reference. Samples were placed in the DSC immediately at a temperature set to 10°C, under nitrogen atmosphere. The temperature was then raised by 2.5°C min^{-1} to 60°C (Dolatowska-Zebrowska *et al.,* 2019). Each run was recorded on the instrument's computer disc. When the run was completed, the temperature measurements were performed using the functions of the Universal Analysis Software (TA Instruments).

3. Results and discussion

3.1. Samples

The subjects of the research were 36 dark (D1 - D36), 23 milk (M1 - M23), 8 white (W1–W8) chocolates, as well as, 2 dark and 2 milk chocolate powder samples (DP1 - DP2, MP1 - MP2).

3.2. Melting characteristics by DSC

Differential scanning calorimetry (DSC) is an important technique used in providing information about processing methods, such as tempering and cooling of the product, as well as the interpretation of the DSC curve on melting characteristic which is the most complex and complicated for evaluation of "quality" in chocolate (Baichoo *et al.,* 2006).

3.3. Melting profiles of fats extracted from dark chocolate samples

The crystalline state and the amount of fat determine the melting properties of chocolate product during the production. The sensory characteristics and storage stability are related to the mechanical and rheological properties of chocolate (Lewicki *et al.*, 2009). Fig.1 illustrated DSC bar charts of melting fats extracted from dark chocolate samples based on their DSC curves (D1 – D36).

Samples and temperature are illustrated on abscissa and ordinate, respectively.

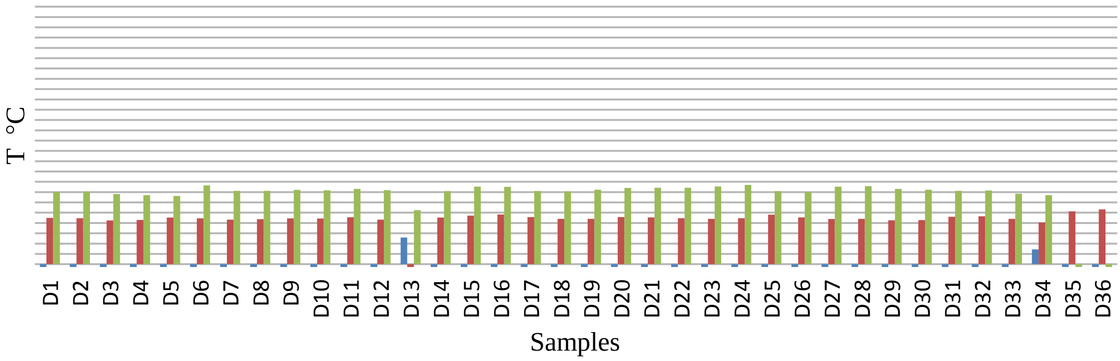

Figure 1. DSC bar charts of melting profiles of fats extracted from dark chocolate samples

In the case of fat extracted from D1 – D36, the maximum temperature of first endothermic peaks were observed at temperature from 18.49°C to 19.63°C and eventually the second distinct, endothermic peaks from 23.40°C to 25.38°C, except D13, D34-D36 samples. The first and second columns may indicate the melting of the crystalline form I of cocoa butter (melting range is 16–18°C) those are illustrated by blue and red, and the third columns present melting of crystalline form II (melting range 22–24°C) those are on green color (Beckett, 2008). The presence of double or multiple peaks in DSC melting curves was often observed in the researches dealing with structures of polymorphic fats (Ostrowska-Ligęza *et al.*, 2019). In 1966, a complete study of the polymorphic states in cocoa butter was conducted and determined the existence of the following polymorphic forms in order of increasing stability: I (sub -α or γ), II (α), III, IV (β'), V (β) and VI. The presence of two or more peaks is attributed to the existence of various crystalline structures within the same product. Moreover, form V (β) makes that cocoa butter remained stable for a very long period of time at the proper storage temperature and was found to have a melting temperature of 33.7–34.9°C (Lipp & Anklam, 1998, 1998a; Merken & Vaeck, 1980). According to Ostrowska-Ligęza *et al.*, (2019) the melting characteristic of fats extracted from dark chocolates is very similar to the melting characteristic of cocoa butter. There were differences in the course of four samples. The appearances of a peak on the curve of fat extracted from dark chocolate D13 were at 15.17 and 20.47°C, D34 were at 12.88, 18.10 and 23.40°C, D35-D36 were at 20.26 and 20.64°C respectively, which conclude that in these cases probably melting of a single crystalline form of fat was observed. Svanberg *et al.* (2011) indicated the effects of major chocolate ingredients and two pre-crystallization techniques on cocoa butter crystallization. The melting characteristic of seeded cocoa butter and sugars had the same course as DSC curves of melting of fats extracted from dark chocolates.

3.4. Melting profiles of fats extracted from milk chocolate samples.

As is known, the main fat phase of milk chocolate contains cocoa butter and milk fat. The DSC bar charts on melting profiles of fats extracted from milk chocolate samples are shown in Fig.2 according to their DSC curves.

Samples and temperature are illustrated on abscissa and ordinate, respectively.

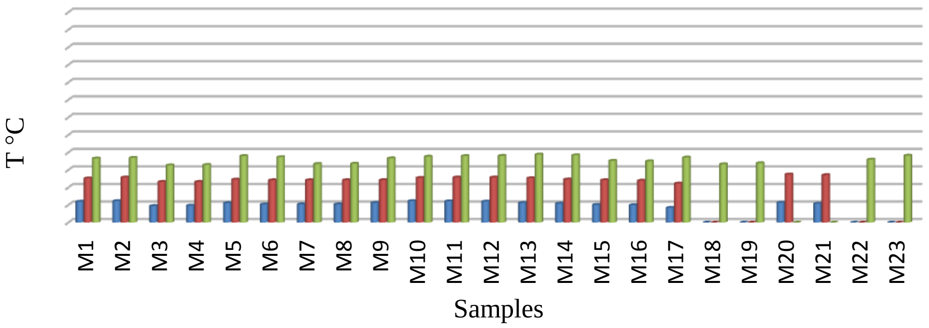

Figure2. DSC bar charts of melting profiles of fats extracted from milk chocolate samples

The DSC diagrams of melting of fats extracted from milk chocolates were characterized by endothermic peaks too. Thus, the maximum temperature of the first endothermic peaks was observed at temperature ranges from 13.36°C to 14.91°C, the second distinct, endothermic peaks from 18.90°C to 20.97°C and eventually, the third mild endothermic peaks from 23.06°C to 25.53 °C, except M18-M23 samples. Milk fat due to its structure is polymorphic fat, and it has a lower melting point than cocoa butter (Ostrowska-Ligęza *et al.,* 2019). The first columns could be related to milk fat which consist of low-melting triacylglycerols content. The saturated and unsaturated fatty acids content in fat affects its melting characteristics (Afoakwa *et al.*, 2008). According to Kim *et al.*, (2009) who studied the thermal properties of milk fat, the distinct peak of melting curves was observed at a temperature of 14 °C, the second and the third endothermic peaks were observed at the temperature of 26.05 and 39.41°C, appropriately. Hence, it can be concluded that, although the second columns may indicate cocoa butter, the third columns which based on the mild endothermic peaks of DSC curves could be the results of overlapping of the peaks derived from milk fat and cocoa butter, accordingly. Besides, milk fat may crystallize taking on three different polymorphic forms: γ, α, and β'. The most stable form of the milk fat is a form of β' and the least stable form is γ (Lopez *et al.*, 2001). As per triacylglycerols content, the form of β' is characterized by an asymmetric structure (fatty acids of various chain lengths, arranged at different positions sn-1 or sn-3). The result of M18, M19, M22 and M23 shows themselves at 23.30, 23.56, 24.38 and 24.27°C, in accordance with the results of overlapping the peaks of endothermic curves of milk fat and cocoa butter. However, M20 and M21 show different melting profiles and temperature range of first distinct was observed at 14.52 and 14.29°C, the second peaks of endothermic curves were determined at 20.97 and 20.84°C which due to the milk and cocoa butter separately.

3.5. Melting profiles of fats extracted from white chocolate samples

Fig.3 illustrated DSC bar chart of melting profiles of fats extracted from white (W1-W8) chocolates samples according to their DSC curves.

Samples and temperature are illustrated on abscissa and ordinate, respectively.

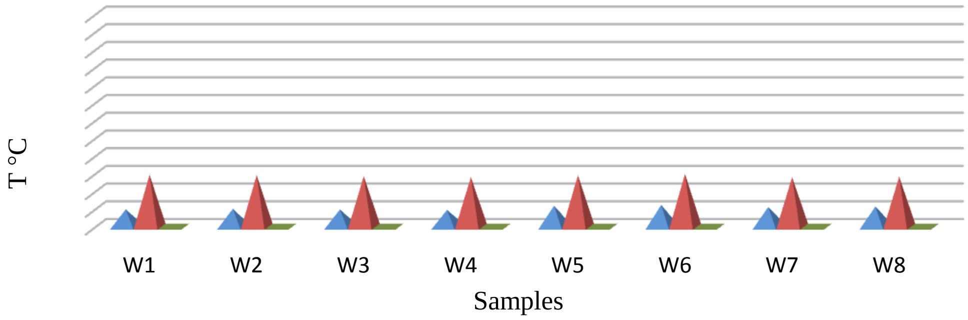

Figure 3. DSC bar charts of melting profiles of fats extracted from white chocolates samples

As usual the DSC melting curves of fat extracted from white chocolate samples (W1 – W8) were characterized by endothermic peaks. Based on DSC curves, the first mild, endothermic peaks were ranged at temperature ranges from 13.81 to 14.93°C, the second distinct, endothermic peaks from 21.21 to 21.94 °C, respectively. There were not observed other endothermic peaks. By comparing these results, it can be explained that due to using milk fat in the production of white chocolate, the melting profiles of white chocolate samples were characterized with low-melting triacylglycerols. Thus, the endothermic peaks may relate to the content of 1st polymorphic form of milk fat and 2nd, also 3rd polymorphic form of cocoa butter. According to Cidell and Alberts (2006), the addition of cocoa butter equivalents (CBE) which have a higher melting point than cocoa butter may affect the melting characteristic of the final product.

3.6. Melting profiles of fats extracted from chocolate powder samples

The DSC bar chart of melting profiles of fats extracted from dark chocolates powder samples (DP1-DP2) and milk chocolate powder samples (MP1-MP2) were described on Fig.4 according to their DSC curves.

Samples and temperature are illustrated on abscissa and ordinate, respectively.

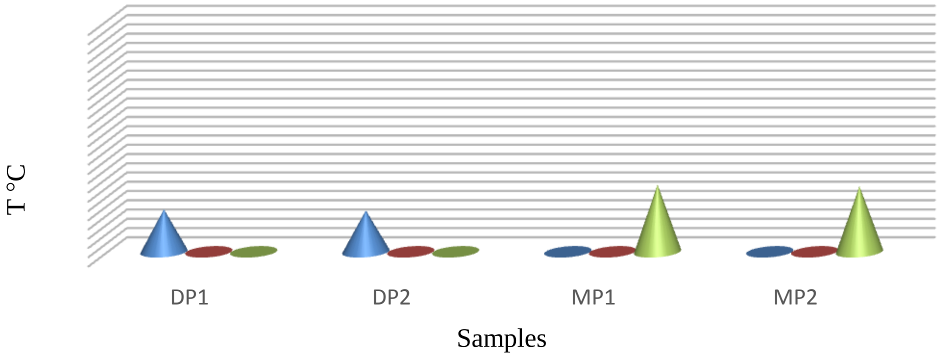

Figure 4. DSC bar charts of melting profiles of fats extracted from chocolate powder samples

It is known that stable cocoa butter crystals will provide chocolate with the following desired properties such as snap, good gloss, proper texture, bloom resistance, contraction for remolding and shelf life after tempering which is carried out under proper temperature and agitation condition (Tanabe & Hofberger, 2006). However, exact temperature and tempering procedures depend upon the equipment and type of chocolate used (Tanabe & Hofberger, 2006). As per the DSC bar charts of melting profiles of fats extracted from chocolate powder based on DSC curves of samples were described in the current experiments. Each sample was characterized by a mild endothermic peak. Thus, the peak of dark chocolate powder samples (DP1-DP2) was at 19.21 and 18.96°C which could be respectively related to the polymorphic forms of cocoa butter. The peak of milk chocolate powder samples (MP1-MP2) following milk fat polymorphic form was at 24.54 and 24.21°C. Although there is not a unique formation to temper chocolate, it confirms that milk chocolate needs lower tempering temperature than dark chocolate (Beckett, 2009).

3. Conclusion

In the overall obtained results on DSC melting curves of fats extracted from the chocolates were observed that there are two endothermic peaks in extracted fats from dark and white chocolate samples and three endothermic peaks in extracted fats from milk chocolate samples. Based on the melting characteristics of fats extracted from milk and white chocolate samples, initial peaks were observed at similar temperatures. The subsequent melting properties of fats extracted from milk chocolate samples were similar to the overall melting characteristic of fats extracted from dark chocolate samples. In addition, the following melting features of fats obtained from white chocolate samples were close to the premier melting characteristic of fats extracted from dark chocolate samples. Naturally, these could be due to the mixing of these two fats and shifting of the melting temperature towards low or high-melting triacylglycerols.

Moreover, the result of DSC melting curves of fats extracted from dark and milk chocolate powder samples are differentiated from each other according to their melting characteristics and were observed an endothermic peak in each sample. Consequently, the results of DSC melting curves of fats from dark chocolate powder samples express lower temperature than the fats extracted from milk chocolate powder samples. It conducts directs the effect of tempering to the fat content of chocolate species. As expected, the result of DSC melting curves of extracted fats from different chocolate samples depends on numerous factors such as the quality of fat and the effect of processing stages of production.

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