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The Oxidative Stability of Fat in Three Dark Chocolates at Different Stages of Manufacturing Process †

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Abstract: The study aimed to compare three dark chocolates and preceding cocoa masses at different stages of production, produced by three different manufacturers using different production methods, based on thermal analysis of fats extracted from mentioned cocoa masses and statistical analysis of the results to assess the impact of production conditions on the oxidative stability of the fat phase. The parameter that allows predicting the sensitivity of the sample to oxidation is the activation energy of the oxidation process. The Ozawa-Flynn-Wall method based on the Arrhenius equation was used to estimate the activation energy (E_a) and the pre-exponential factor (Z).The measurements were carried out using DSC apparatus (Q20, TA Instruments) and non-isothermal mode with the following sample heating rates: 2.5 K/min, 4 K/min, 6 K/min, 7.5 K/min, 10 K/min, 12.5 K/min, 15 K/min. An increase in the activation energy corresponding to the increase in fat oxidative stability was observed in the case of raw dark chocolate from a small company and classic dark chocolate from a large company. In the case of classic dark chocolate produced by a small manufacturer, the stability of fat in the final product was much lower (65.36 kJ/mol) than that of the starting material such as cocoa butter (123.89 kJ/mol) or cocoa liquor (104.68 kJ/mol).

Keywords: DSC; cocoa butter; oxidative stability

1. Introduction

Differential scanning calorimetry (DSC) is a simple, fast and effective method to characterize material properties such as glass transition temperature, melting, crystallization, specific heat capacity, cure process, purity, oxidation behavior and thermal stability. The difference between the heat flow rate of the test sample and known reference material determines variations in material composition, crystallinity, and oxidation. The heat released from a particular reaction using DSC can be registered in either isothermal or non-isothermal mode. In general, non-isothermal methods are more useful to assess the lipid resistance to oxidation, which is a free-radical chain reaction that leads to undesirable taste and smell [1]. DSC analysis provides also crucial kinetic information especially about initiation step of oxidation reaction.

The non-isothermal method is based on the linear correlation between temperature, which can affect specific thermal event, and a different heating rate [2]. From the Arrhenius like equation the effective activation energy (Ea) of oxidation and pre-exponential factor (Z) are calculated [3].

This work aimed to study thermooxidation of the fat phase during different chocolate production processes based on three local manufacturers. The results could be taken as an indication ofthe validity of the parameters used in the production as well as the suitability of the produced chocolates for baking.

2. Materials and Methods

2.1. Materials

Samples of chocolate and chocolate masses from a complete production line from three different producers were obtained courtesy of three anonymous companies.

2.2. Cocoa Butter Extraction from Chocolates and Cocoa Masses

Cocoa butter was extracted from cocoa beans according to the procedure described by Boselli et al. [4]. Approximately 30 g of the sample was homogenized 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. Additional 100 mL of chloroform was added and the content was homogenized for 2 min. The mixture was filtrated to separate any solid residue and 70 mL of 1 M KCl solution was added to filtrate. The liquid was left overnight at 4 °C in order to phase separation. The organic phase was collected and the solvent was removed by the rotary evaporator at 40 °C. The cocoa butter sample was stored at −18 °C until it was analyzed.

2.3. DSC Measurements

The non-isothermal (dynamic) mode of DSC was used. The samples and reference pans were heated from 303 to 623 K at the rates of 2.5, 4, 6, 7.5, 10, 12.5 K/ min. The experiments were performed in an atmosphere of oxygen with an initial pressure of 101kPa and the gas flowing at a rate of 50 mL/min. For each programmed heating rate (β, K/min), determinations were carried out three times. The onset oxidation temperature (T_{on}, K) was determined as the intersection of the extrapolated baseline and the tangent line (leading edge) of the recorded exotherm. The kinetic parameters of the oxidation process (activation energy and pre-exponential factor) were calculated.

3. Results

Onset oxidation temperatures (T_{on}) values obtained for three different chocolates and their masses at different stages of production process were used as primary parameters for the assessment of the resistance of tested fats to their thermal oxidative decomposition. The processed data given in Tables 1–3 show, that the fat from classic dark chocolate produced by small manufacturers is characterized by a lowest activation energy in a final chocolate bar. In this production process it is also observed the biggest decrease in oxidative stability of fat comparing cocoa butter as starting material and final product—chocolate bar. Similar but less significant drop in oxidative stability is observed for raw dark chocolate produced by small manufacturer. These findings are in opposition to data obtained for classic dark chocolate produced by large manufacturer—cocoa butter used for chocolate production has significantly smaller oxidative stability than the final chocolate bar. The production process in case of this company is long multi-step process accompanied by addition of an emulsifier declared on the label. Emulsifiers are usually rich in mono- and polyunsaturated fatty acids which naturally prevent thermoxidation of the mixture [5]. Despite the use of high temperature during production process, the fat from chocolate is more stable than the fat from chocolates which were treated more gently during production procedure. During thermal food processing melanoidins are produced, which are suggested to have antioxidant properties [6].

Table 1. Regression analysis of DSC data, activation energies (E_a) and pre-exponential factors of oxidation reaction of fat phase extracted from chocolate masses during raw dark chocolate production process, small manufacturer.

Parameter	Cocoa Butter	Cocoa Liquor	Tempered Mass	Chocolate
a	6953	8250	6997	5341
b	15.02	18.08	15.23	12.48
R^2	0.999	0.997	0.999	0.991
E_a/kJ mol^{-1}	126.58 [1]	150.19 [1]	127.38 [1]	97.24 [1]
Log Z	13.15	16.14	13.36	10.73
Z/min^{-1}	1.42×10^{13}	1.37×10^{16}	2.29×10^{13}	5.33×10^{10}

[1] Calculations based on onset temperatures.

Table 2. Regression analysis of DSC data, activation energies (E_a) and pre-exponential factors of oxidation reaction of fat phase extracted from chocolate masses during classic dark chocolate production process, small manufacturer.

Parameter	Cocoa Butter	Cocoa Liquor	Conched Mass	Chocolate
a	6805	5750	5510	5166
b	14.82	13.21	12.80	10.78
R^2	0.996	0.990	0.990	0.960
E_a/kJ mol^{-1}	123.89 [1]	104.68 [1]	100.31 [1]	65.36 [1]
Log Z	12.96	11.42	11.03	7.15
Z/min^{-1}	9.16×10^{12}	2.66×10^{11}	1.08×10^{11}	1.40×10^{7}

[1] Calculations based on onset temperatures.

Table 3. Regression analysis of DSC data, activation energies (E_a) and pre-exponential factors of oxidation reaction of fat phase extracted from chocolate masses during classic dark chocolate production process, large manufacturer.

Parameter	Cocoa Butter	Cocoa Liquor	Conched Mass	Chocolate
a	4528	5876	5768	6682
b	10.77	13.34	14.24	15.16
R^2	0.998	0.998	0.989	0.989
E_a/kJ mol^{-1}	82.43 [1]	106.98 [1]	105.01 [1]	121.65 [1]
Log Z	9.09	11.55	12.45	13.31
Z/min^{-1}	1.23×10^{9}	3.51×10^{11}	2.84×10^{12}	2.04×10^{13}

[1] Calculations based on onset temperatures.

The Equations (1)–(3) were used to calculate activation energy and pre-exponential factor with an assumption that the degree of oxidation reaction is a constant value independent of the heating rate,

$$\text{Log}\beta = a(1/T_{on}) + b \quad (1)$$

$$E_a = -2.19\ R\ [d\ \text{log}\beta/d(1/T_{on})] \quad (2)$$

$$Z = \beta E_a\ e^{(Ea/RT)}/RT^2 \quad (3)$$

where β is the heating rate (K/min) and T is the temperature, a and b are the slope and intercept from Equation (1), respectively, Ea is effective activation energy and R is the universal gas constant (8.31 J/mol K). In non-isothermal oxidation of lipids, the consumption of oxygen can be neglected due to the large excess of oxygen generated by a constant flow rate. Such condition allows the formation of peroxides, being independent of the oxygen concentration, therefore the autoxidation is a first order reaction.

4. Discussion

Cocoa butter is characterized in literature by very high kinetic parameters (E_a, Z) dependent on many factors such as sample pre-treatment, sample preparation, sample size, heating rate and DSC mode [4]. Ostrowska-Ligeza et.al determined activation energy of cocoa butter in non-isothermal mode $Ea_{(Ton)}$ = 140.93 kJ/mol [7]. Cifti et al. applied isothermal mode in their studies and obtained lower activation energy *Ea* = 106.2 kJ/mol [8]. In this paper cocoa butter in either pure form or as a main ingredient of extracted from chocolate masses fat phase showed a wide range of activation energies *Ea* = 65.36 − 150.19 kJ/mol. A similar tendency was shown for the calculated pre-exponential factor *Z*. With such a wide dispersion of the results in the literature, one suspects less obvious reasons for the oxidative stability of cocoa butter during chocolate production. In multicomponent systems, the fat sometimes needs to be extracted from the matrix. Therefore, the lipid oxidative behaviour might be different from its original matrix. The results obtained from DSC oxidation depend upon the conditions used to prepare the sample and the heating protocol used. Factors, such as degree of saturation, amount of free fatty acids, chain length and the presence of natural antioxidants influence the oxidative stability and kinetic parameters. Moreover, *Ea* should not be used as a single parameter to rank the oxidative stability of lipid systems [9] because interpretation of *Ea* varies within the Arrhenius principle: oil with a high *Ea* value oxidizes faster at high temperatures, while oil with a low *Ea* value oxidizes faster at low temperatures [5]. Bearing in mind the above considerations, the results obtained in this research are difficult to compare with other studies conducted for cocoa butter. They provide unique information for production process of each chocolate separately, as well as they allow to identify the actual moment of the production process when the quality of the fat in the chocolate deteriorates.

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