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Comparison of Thermal Characteristics and Fatty Acids Composition in Raw and Roasted Cocoa Beans from Peru (Criollo) and Ecuador (Forastero)

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Abstract: The aim of this research was to complete the characteristics of cocoa beans and cocoa butter extracted from two different *Theobroma cacao* species: Criollo originated from Peru and Forastero originated from Ecuador, both in the version of raw (unroasted) and roasted beans. Before extraction, the cocoa beans were characterized by proximate analysis. The determination of fatty acids composition was carried out by gas chromatography (GC). The positional distribution of fatty acids in the sn-2 positions of triacylglycerols (TAGs) was also determined. The thermogravimetric analyses (TGA/DTG) were performed under the nitrogen and oxygen atmosphere of roasted and unroasted cocoa beans. The kinetic information was helpful to assess the oxidative stability of cocoa butter. The cocoa butter extracted from unroasted Forastero from Ecuador had the highest values of oxidation activation energy E_a. The melting characteristics of cocoa butter extracted from roasted Criollo species were very similar to their unroasted versions. The same trend was not observed for Forastero species. TGA and DTG were revealed to be useful tools for the analysis of whole cocoa beans and the fats extracted from these cocoa beans.

Keywords: cocoa beans; cocoa butter; TGA; DSC; GC

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1. Introduction

Cocoa butter (CB) is one of the most precious and useful vegetable fat obtained from cocoa beans. Due to its unique properties such as pale yellow colour, neutral taste, and sharp melting profile, body-temperature alike, it is widely used in both cosmetics and food preparation. The functionality of this fat is mainly a consequence of the predominant presence of symmetrical triacylglycerols (TAGs) [1]. CB consist mostly of saturated fatty acids such as stearic acid (C18:0) and palmitic acid (C16:0) and about 30–35% of unsaturated fatty acids. The fatty acids (FA) profile of cocoa butter may slightly vary for different species of cocoa plants and different geographical origins. Normally, the nearer the equator the cocoa was grown, the harder will be the fat obtained [2]. The quantitative analysis of FA composition is essential in food research with regards to the nutritional value content and purity or authenticity of fats [3].

The fermentation, drying, transportation and storage are four operational steps, compulsory upon all the cocoa beans during the cocoa butter production [4]. They take place according to the optimized conditions which contribute to the desired colour and flavour of resulting fat [5]. Roasting however is an optional, but not essential, step during the cocoa beans processing. It is applied as additional protection against microbiological contaminants as well as flavour changing factors. Cocoa butter can be derived from either roasted or unroasted cocoa beans [6,7].

Although cocoa butter is a relatively simple mixture of triacylglycerols, it represents very complicated polymorphism. It can crystallize as a function of TAGs composition into six polymorphic forms (I-VI), where form I is the least stable and form V is the most desirable but it can easily change into form VI, the most stable one, in storage [8]. Differential scanning calorimetry (DSC) is a simple, fast and effective method to characterize changes in fat melting profile and measure the relative amounts of each crystalline state. The peaks corresponding to latent heat are observed in temperature ranges related to the melting of specific polymorphs [9]. 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 [10]. DSC analysis provides also crucial kinetic information especially about the initiation step of the oxidation reaction. Gouveia et al. [11] suggested Temperature Modulated Optical Refractometry (TMOR) for cocoa butter thermal transitions characterization. The transition temperatures obtained by TMOR agreed well with DSC results with a systematic variation of 2 °C. Additionally, a third thermal event was detected by TMOR, while no counterpart was found on the DSC result [11].

The non-isothermal method is based on the linear correlation between temperature, which can affect specific thermal events, and a different heating rate [12]. From the Arrhenius-like equation, the effective activation energy (E_a), pre-expotential factor (Z) and constant rate (k) are calculated [13].

Thermogravimetry (TGA) and derivative thermogravimetry (DTG) are very fast, cost-effective, and simple analytical methods to determine the thermal stability of materials, the composition of complex mixtures and the kinetics of their decomposition. By far it has been successfully used to control the quality of fuels, ceramics, polymers, absorbents, pharmaceuticals and food. The application of TGA in the thermal analysis provides unique information about the nature of the sample and the modifications induced by the industrial processing [14] such as drying or roasting.

The higher than the mostly occurring 1–2% amount of trisaturated triacylglycerols (containing saturated fatty acids in all positions) in cocoa butter increases its melting point. However, when the cocoa butter is melted and then cooled, differently composed triacylglycerols behave not alike. The trisaturated TAGs crystallize first, which makes the final product thicker in consistency, whereas monounsaturated TAG with oleic acid in sn-2 position crystallize later and determine the resistance of the chocolate to fat bloom [2,15]. Moreover, the distribution of fatty acids in unroasted cocoa beans of two different origins was analysed by Torres-Moreno et al. [16]. Their results confirmed that the geographical origin had an influence on the fatty acid composition of cocoa butter.

The aim of the present study was to characterize thermal properties, oxidative stability and other compositional aspects of the cocoa butter extracted from roasted and unroasted cocoa beans of two different species: Criollo derived from Peru and Forastero from Ecuador.

2. Materials and Methods

2.1. Samples

Theobroma cacao beans of two types and two geographical origins, Peru (Criollo) and Ecuador (Forastero), were purchased from one supplier already in a roasted and unroasted form of beans. The cocoa beans were classified into two kinds: bulk beans (Forastero) and fine flavour beans (Criollo). Forastero species account for about 85% of the world's cocoa harvest. Criollo species accounts for about 3% of the world's cocoa harvest. Forastero cocoa bean is characterized by a dark-brown color, a slightly stronger aroma than Criollo. It is slightly bitter and contains more fat. Criollo cocoa bean is characterized by a bit lighter color and a mild, nutty aftertaste ([17]; Ławrowski 2018).

The harvest of cocoa beans Criollo and Forastero took place in 2017.

2.2. Cocoa Butter Extraction from Cocoa Beans

Cocoa butter was extracted from cocoa beans according to the procedure described by Boselli et al. and Wirkowska-Wojdyła et al. [18,19]. The Folch method with the use of a mixture of chloroform and methanol as solvents was used.

2.3. Proximate Analysis

The roasted and unroasted cocoa beans were analysed in duplicate for moisture, ash, protein, total fat and mineral composition in triplicate in the accredited Analytical Centre (accreditation certificate no PCA AB439) at Warsaw University of Life Sciences.

Total carbohydrate content was calculated by the difference after analysing other components.

Moisture contents of the samples were determined by a gravimetric method by drying 2 ÷ 2.3 g of the ground sample at 105 °C ± 1 °C to constant mass in an air oven. Ash contents were determined by placing a 2 g sample in a muffle furnace at 550 °C for 20 h. Fat was determined in a Soxhlet apparatus using petroleum ether as the solvent of extraction. Total organic nitrogen was determined by using the macro Kjeldahl procedure. The protein content of samples was calculated using 6.25 as the conversion factor (Protein = Nitrogen * 6.25).

The mineral composition of unroasted and roasted cocoa samples was analysed in a microwave digestion (Milestone 1200 MEGA, 220 °C; HNO_3:H_2O_2, 8:1 v/v) was used to eliminate organic matter before 1 g samples were analysed. Calcium, Iron, Potassium, Magnesium, and Phosphorus were all determined and analysed using the ICP-AES method (ThermoiCAP 6500 DUO). Calibration was made using a Certified Reference Material (CRM) ERM-BD 150 (Skimmed Milk Powder). The accuracy of the measurements was confirmed by high recovery percentages which were 94%, 86%, 93%, 99% and 103% for Calcium, Iron, Potassium, Magnesium, and Phosphorus respectively.

2.4. Determination of Fatty Acid Composition

The determination of fatty acid composition was carried out by gas chromatography (GC) analysis of fatty acids methyl esters according to the procedure described by Wirkowska-Wojdyła et al. [19]. Measurements were done in triplicate. The identification of fatty acids was carried out using the lipid standard purchased from Sigma Aldrich (Sulpeco 37 Comp. FAME Mix 10 mg/mL in CH_2Cl_2) [19].

2.5. Sn-2 Positional Fatty Acid Analysis by Pancreatic Lipase

The positional distribution of fatty acids in the sn-2 and sn-1,3 positions of triacylglycerols was determined according to the method described by Pina-Rodriguez, Akoh [20] and Yüksel, ŞahinYeşilçubuk [21].

2.6. DSC Measurements

The calorimetric measurements were performed with a Q200 DSC (TA Instruments, New Castle, DE, USA). The instrument was calibrated in temperature and enthalpy with high purity indium according to the procedure for standard DSC. Integration and onset temperature measurements were performed using the functions of the Universal Analysis Software (TA Instruments).

2.6.1. DSC Study of Cocoa Bean Powders

Powdered cocoa beans samples (10–15 mg) were hermetically sealed in aluminium pans and heated from −60 to 300 °C at a heating rate of 5 °C/min. The nitrogen flow through the cell was 50 mL/min. The empty identically closed aluminium pan was the reference crucible [22].

2.6.2. DSC Kinetics Parameters

Oxygen was used as the purge gas at a rate of 50 mL/min. Fat (cocoa butter) samples of 3–4 mg were placed in aluminium sample pans and inserted into the heating chamber of

the DSC cell. The aluminium reference pan was left empty. Samples were heated in an open aluminium pan with linear heating rates of 2.5, 4, 5, 7.5, 10, 12.5 and 15 °C/min. For each programmed heating rate (β, °C/min), at least triplicate determinations were carried out. When the run was completed, the onset oxidation temperature (T_{on}, °C) was determined as the intersection of the extrapolated baseline and the tangent line (leading edge) of the registered exotherm. The averages from measurements of T_{on} for each cocoa butter sample at a given temperature were determined as the intersection of the extrapolated baseline and the tangent line (leading edge) of the registered exotherm [23].

2.6.3. Melting Characteristics

Fat samples of 3–4 mg were placed into aluminium pans with a lid and were hermetically sealed. An empty sealed aluminium pan was used as a reference and the experiments were performed under a nitrogen normal pressure flowing with a rate of 50 mL/min. Melted samples were heated to 80 °C and held for 10 min, in order to melt all the crystals and to erase the thermal memory. The samples were then cooled to −80 °C at 10 °C/min and maintained at −80 °C for 30 min. Then the melting (so-called second fusion) profiles were obtained by heating the samples to 80 °C at a heating rate of 15 °C/min [23].

2.7. Thermogravimetry Analysis

The thermogravimetry curves were obtained by using a Discovery TGA (TA Instruments, New Castle, DE, USA). The flow rate of nitrogen or oxygen was 25 mL/min. The samples (powdered cocoa beans and cocoa butter) within the mass 7–8 mg, were placed in platinum containers. The results were obtained in triplicate, within temperature range 50–700 °C, and at a scanning rate of 10 °C/min. The fluctuations between trials were below 5% [14,24].

2.8. Statistical Analysis

The data were reported as the means ± standard deviation. One-way ANOVA was performed using the Statgraphics Plus, Version 5.1 (Statistical Graphics Corporation, Warrenton, VA, USA). Differences were considered to be significant at a *p*-value of 0.05, according to Tukey's Multiple Range Test. The experiments were carried out in three replications.

3. Results

3.1. Chemical Composition of Unroasted and Roasted Cocoa Beans Criollo and Forastero

The chemical composition of the unroasted and roasted cocoa beans from Peru (Criollo) and Ecuador (Forastero) is shown in Table 1. For both types, fat was the major nutrient (>40%), followed by carbohydrates (29–35%) and proteins (14.1–14.8%). Ash and moisture contents were less than 6% each. Unroasted and roasted cocoa bean composition varied significantly only in moisture content, 5.7 and 3.1 g per 100 g of the sample, respectively. The values found for proteins and total fat were different but comparable to those obtained by Torres-Moreno et al. [16] when studying differences in fatty acids profile in cocoa beans with different geographical origin. Results related to the mineral composition of unroasted and roasted cocoa beans, as shown in Table 1, indicated that cocoa beans contain several minerals which play a major role in different body functions including enzymatic reactions, energy production and transmission of nerve impulses [25,26]. Significant differences due to the effect of roasting were found for iron, especially for Forastero species. Both cocoa beans were planted on the soil with comparable parameters.

Table 1. The chemical composition of roasted and unroasted cocoa bean samples from Ecuador and Peru.

Ecuador Cocoa Beans Forastero Type		**Unroasted**	**Roasted**
Proximates% (g/100 g)	Moisture	5.70 ± 0.34 *,a	3.1 ± 0.19 b
	Ash	3.96 ± 0.24 a	4.06 ± 0.24 a
	Total protein	14.10 ± 0.71 a	14.60 ± 0.73 a
	Carbohydrate and fibre (by difference)	29.54 ± 6.50 a	32.74 ± 7.20 a
	Total fat	46.70 ± 3.27 a	45.50 ± 3.19 a
Minerals	Calcium, Ca (mg/kg)	1598 ± 320 a	1479 ± 296 a
	Magnesium, Mg (mg/kg)	3629 ± 726 a	3547 ± 709 a
	Potassium, K (%)	1.26 ± 0.25 a	1.30 ± 0.26 a
	Phosphorus, P (mg/kg)	5247 ± 1050 a	5150 ± 1030 a
	Iron, Fe (mg/kg)	53.6 ± 10.7 a	135 ± 27.0 b
Peru Cocoa Beans Criollo Type		**Unroasted**	**Roasted**
Proximates% (g/100 g)	Moisture	5.50 ± 0.33 *,a	4.90 ± 0.29 a
	Ash	3.53 ± 0.21 a	3.38 ± 0.20 a
	Total protein	14.62 ± 0.73 a	14.80 ± 0.74 a
	Carbohydrate and fibre (by difference)	35.35 ± 7.78 a	35.02 ± 7.70 a
	Total fat	41.00 ± 2.87 a	41.90 ± 2.93 a
Minerals	Calcium, Ca (mg/kg)	1002 ± 200 a	1008 ± 202 a
	Magnesium, Mg (mg/kg)	3648 ± 729 a	3524 ± 705 a
	Potassium, K (%)	1.06 ± 0.21 a	1.10 ± 0.22 a
	Phosphorus, P (mg/kg)	5933 ± 1190 a	5579 ± 1116 a
	Iron, Fe (mg/kg)	38.9 ± 7.8 a	55.0 ± 11.0 b

* Stated as total absolute uncertainty of the result obtained. Values with different letters within a row are significantly different ($p < 0.05$) according to Tukey's test.

Cocoa is currently cultivated in most of the tropical lowlands throughout the world. Cocoa plantations are mostly suitable within 20° north and south of the Equator. An altitude between 200 m and 300 m is also suitable for cocoa cultivation. Cocoa requires a constant supply of rainfall that is evenly distributed throughout the year; preferably between 1500 mm to 2000 mm. Cocoa cultivation also requires temperatures between 18 °C and 32 °C. The soil conditions prevailing on cocoa plantations also require that they are porous, well-drained and more importantly rich in humus and other vital minerals needed for its growth. In effect, the soil should be fertile. Soil conditions exhibiting a pH between neutral and slightly acidic are also preferred, whilst carefully controlling the prevalence of pests and diseases ([27]; Fowler, 1999). Cocoa trees may grow to a height of about 3 m or even more depending on the variety ([28]; Dissertation Berbiye 2014, 17; Ławrowski 2018).

Therefore the most probable explanation for this difference is the roasting procedure which always takes place in a metal container, so-called batch. Thermal treatment of the beans with the residues of acetic acid and lactic acid after the fermentation process and the presence of hot air together may cause the metal batch, mostly made of steel, to release traces of iron and other metals during the roasting process [29,30].

3.2. Composition and Distribution of Fatty Acids of Cocoa Butter Extracted from Unroasted and Roasted Cocoa Beans Criollo and Forastero

Fatty acids from cocoa butter derived from roasted and unroasted cocoa beans were identified and quantified using GC-FID analyses, showing the separation of nine different fatty acid methyl esters (FAME) stated in Table 2.

Table 2. The composition of the selected fatty acids and their distribution in triacylglycerols in analysed cocoa butter from roasted and unroasted cocoa beans from Ecuador and Peru.

Samples	Fatty Acid	Fatty Acid Composition in TAG	Fatty Acid Composition (%) in Positions		The Share of the Fatty Acid in sn-2 Position (%)
			sn-2	sn-1,3	
Ecuador (Forastero) Roasted	C16:0	27.84 ± 0.63	21.91 ± 0.44	30.81	23.71
	C18:0	34.46 ± 0.45	25.83 ± 0.18	38.78	22.21
	C18:1n-9c	32.09 ± 0.13	47.40 ± 0.78	24.44	64.66
	C18:2n-6c	3.43 ± 0.03	4.87 ± 0.53	2.71	59.78
	C20:0	1.31 ± 0.08	-	1.97	-
Ecuador (Forastero) Unroasted	C16:0	30.02 ± 1.46	10.57 ± 0.13	39.75	11.74
	C18:0	33.54 ± 1.63	12.99 ± 0.04	43.82	12.91
	C18:1n-9c	32.76 ± 0.68	69.90 ± 0.18	14.19	71.12
	C18:2n-6c	2.50 ± 0.02	5.63 ± 0.01	0.94	75.07
	C20:0	1.04 ± 0.02	-	1.56	-
Peru (Criollo) Roasted	C16:0	27.11 ± 0.87	3.03 ± 0.01	39.14	3.73
	C18:0	33.12 ± 0.46	2.76 ± 0.01	48.29	2.78
	C18:1n-9c	33.48 ± 0.33	81.35 ± 0.08	9.54	81.00
	C18:2n-6c	3.54 ± 0.04	9.55 ± 0.14	0.53	90.05
	C20:0	1.30 ± 0.07	-	1.95	-
Peru (Criollo) Unroasted	C16:0	28.03 ± 1.55	3.52 ± 0.08	40.28	4.18
	C18:0	33.44 ± 0.91	3.28 ± 0.01	48.51	3.27
	C18:1n-9c	32.44 ± 0.46	80.51 ± 0.08	8.40	82.73
	C18:2n-6c	3.28 ± 0.06	8.70 ± 0.06	0.56	88.55
	C20:0	1.38 ± 0.11	-	2.07	-

The means ± standard deviation.

The properties of fats depend not only on the composition of fatty acids but also on their distribution within triacylglycerols. The position of fatty acids in TAG molecules is especially important during digestion and absorption in the human body [10].

Fatty acids detected in roasted and unroasted cocoa beans were: C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C17:0 (margaric acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (linolenic acid) and C20:0 (arachidic acid) ranked in order of the retention time. For unsaturated fatty acids, both, position and Z/E geometrical isomerism for the double bond were differentiated based on FAME standard. The distribution of main fatty acids between triacylglycerols positions in cocoa butter from roasted and unroasted cocoa beans is given in Table 2. In all samples, the roasting process influenced the composition and distribution of fatty acids insignificantly. The amount of linoleic acid increased in the case of both species. These changes were more evident for Forastero samples. The oleic acid was predominantly esterified in the internal position (sn-2) of the TAG of all cocoa butter samples.

SFA accounted for 62% in the case of Criollo species and 64% in Forastero by weight and they were mostly esterified in the external positions of the TAG. Such a distribution of fatty acids decreases the absorption of fatty acids in the intestinal lumen. Lipolysis of triacylglycerol by pancreatic lipase occurs mainly in the sn-1 and sn-3 positions, yielding free fatty acids and a 2-monoacylglycerol. Free stearic acid, comparable to free palmitic acid, is likely to form insoluble complexes with calcium and magnesium ions which cannot be absorbed well in the intestine. Hence, in the form of insoluble salts, they are excreted in the feces, leading to the loss of both, energy source and important macroelements such as magnesium and calcium [31–33].

3.3. DSC Study of Beans and Cocoa Butter Extracted from Unroasted and Roasted Cocoa Beans Criollo and Forastero

DSC curves of the whole beans from Forastero and Criollo species in two forms: roasted and unroasted (Figure 1) were significantly different especially considering the

second thermal transition referring to carbohydrates. The first thermal transition was similar for all forms and ranged from 9–43 °C. The first peaks on powdered cocoa beans DSC curves were characteristic of the cocoa butter that is an ingredient of cocoa beans. Agus et al. [34] studied the composition of unroasted and roasted cocoa beans from Peninsular Malaysia. The fat content of unroasted cocoa beans was significantly higher than that of roasted cocoa beans. In roasted and unroasted cocoa beans, the content of fat is relatively high and the first peak corresponding to fat transitions on all DSC curves was present. The second transition was different in the case of all forms. The maximum peak for Criollo was present at temperatures of 140.9 and 175.04 °C for roasted and unroasted beans, respectively. In the case of Forastero species, the second transition at 175.05 and 181.30 °C listed in the same order was observed. According to Agus et al. [34], the carbohydrate content during the roasting process is changed. The reactions most affecting the composition of cocoa beans are caramelization and Maillard reactions [35]. Redgwell et al. [36] investigated the roasting of three varieties of cocoa beans in different parameters. The concentrations of glucose and fructose decreased after roasting but levels of the non-reducing sugars, sucrose, raffinose, stachyose and verbascose were not markedly affected [36].

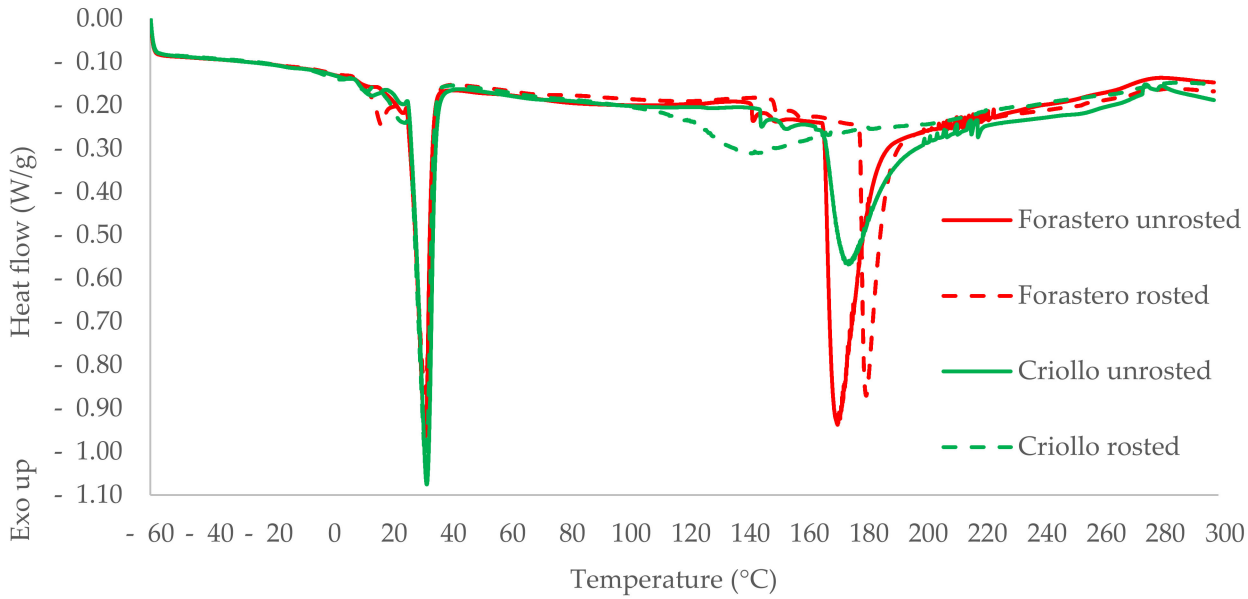

Figure 1. Differential scanning calorimetry (DSC) curves of ground, roasted and unroasted, Criollo (Peru) and Forastero (Ecuador) powdered cocoa beans.

The melting characteristics of the cocoa butter fractions were studied by the means of DSC which provides a generalized view of the melting of the fat fraction over its melting range (melting profile). The shapes of DSC melting curves of the cocoa butter from roasted Criollo cocoa beans were similar to unroasted ones (Figure 2). Both exhibited narrow melting ranges from 15 to 21 °C and two endothermic, distinct peaks. Azir et al. [3] studied the addition of lard to cocoa butter through changes in fatty acids composition, triacylglycerols profile, and thermal characteristics. The melting temperature of cocoa butter was similar and reached the value of about 18 °C. In the case of fat extracted from unroasted Criollo cocoa beans lower first melting point but higher second melting point than from roasted ones was detected. DSC profile for fat extracted from Forastero unroasted cocoa beans differed from roasted one and was characterized by four endothermic peaks at maximum temperature −10.50, 1.50, 9.12 and 18.84 °C. In the case of Forastero species, the roasting process influenced the shape of the curve at the DSC diagram of the fat extracted from roasted and unroasted cocoa beans. The analysis of course of cocoa butter melting curve showed the presence of four endothermic peaks [3]. The saturated and unsaturated fatty acids content affects the melting characteristics of analysed fat. The increased amount

of long-chain saturated fatty acids usually results in a greater proportion of high-melting triacylglycerols and higher melting points. Considering obtained DSC melting curves for the fats derived from both roasted and unroasted Forastero cocoa beans, it can be concluded that both fats have comparable fatty acid profiles. These dependencies were not observed for Criollo species.

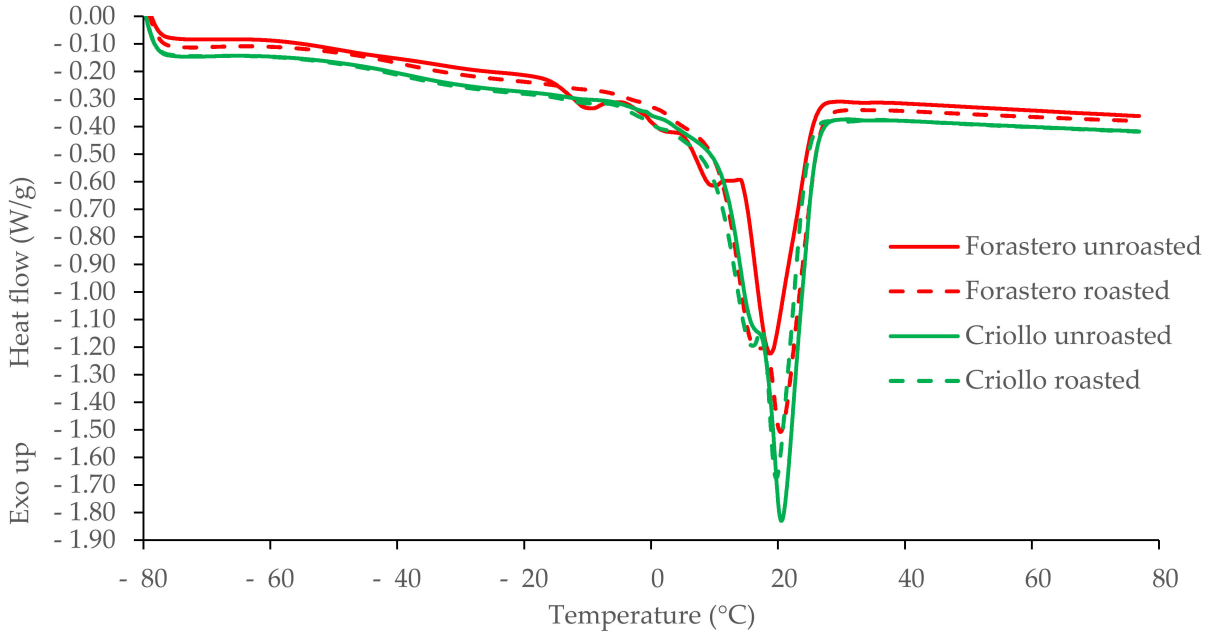

Figure 2. DSC melting curves of fats extracted from roasted and unroasted, Criollo (Peru) and Forastero (Ecuador) cocoa beans.

3.4. DSC Study of Cocoa Butter Extracted from Unroasted and Roasted Cocoa Beans Criollo and Forastero

Table 3 shows the experimental onset oxidation temperatures (T_{on}) obtained at seven heating rates (2.5, 4, 5, 7.5, 10, 12.5 and 15 °C/min). As the heating rate increases, the T_{on} increases as well. This can be described by the following linear correlation (Equation (1)):

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

where β is the heating rate, a and b are adjustable coefficients and T_{on} is the onset oxidation temperature.

Table 3. DSC heating rates (β) and oxidation onset temperatures (T_{on}) for cocoa butter extracted from cocoa beans.

Heating Rate, β/°C	Oxidation Onset Temperatures T_{on}/°C			
	Forastero Unroasted	Forastero Roasted	Criollo Unroasted	Criollo Roasted
2.5	188.08 ± 1.90	185.44 ± 2.08	185.61 ± 0.04	195.82 ± 0.11
4	195.35 ± 0.93	189.85 ± 1.70	192.38 ± 0.01	201.53 ± 0.49
5	196.64 ± 0.34	194.13 ± 1.27	195.75 ± 0.96	205.36 ± 0.45
7.5	203.35 ± 1.61	199.32 ± 1.63	202.92 ± 0.40	210.46 ± 0.84
10	208.44 ± 1.36	203.97 ± 2.24	207.77 ± 0.02	217.20 ± 0.06
12.5	210.88 ± 1.08	209.11 ± 0.25	212.88 ± 0.53	220.05 ± 0.03
15	213.43 ± 0.54	210.83 ± 0.39	213.99 ± 0.33	222.34 ± 0.73

Values represent means ± standard deviations.

During slow heating, primary oxidation intermediates are produced such as hydroperoxides which react with an excess of oxygen to form low molecular weight compounds such as aldehydes and acids that remain in solution and accelerate the degradation pro-

cess [37,38]. At fast heating rates, in contrast, these intermediate products are lost through evaporation before they can further react with the melted fat, hence they do not shift distinctly the start of the DSC peak to higher values. The T_{on} values obtained for each fat can be used as a primary parameter to assess the resistance of the tested cocoa butter isolated from two different species to its thermal decomposition. Generally, the higher the onset temperature, the more stable is the fat. The fat obtained from unroasted cocoa beans was more stable in the case of Forastero species than Criollo species as the values of T_{on} for all the heating rates were significantly higher. Unlike the above, the fat from roasted Criollo cocoa beans showed better thermal stability than the corresponding fat from Forastero species. This trend was difficult to capture based on DSC melting curves which look very similar for all analysed fats. The effect of the roasting procedure on the stability of fats within the beans is ambiguous. For Criollo type of beans, the T_{on} values increased after roasting whereas the Forastero beans showed the opposite tendency.

In the DSC method which is based on the recording of released heat in non-isothermal mode, the consumption of oxygen can be neglected due to the large excess of oxygen generated by a constant flow rate. Therefore the auto-oxidation is a first-order reaction. This fact can be used to calculate kinetic parameters such as activation energy (E_a), pre-exponential factors (Z) and reaction rate constant (k). Using the information presented in Table 3 and the Equations (2)–(4):

$$E_a = -2.19\,R\,\frac{d\,\log\beta}{d(1/T)} \quad (2)$$

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

$$k = Z\exp\left(-E_a/RT\right) \quad (4)$$

where R is a gas constant, β is the heating rate (°C/min) and T is temperature (K), the kinetic parameters were calculated and the values were presented in Table 4.

Table 4. Regression analysis of the DSC data, activation energies (E_a), pre-exponential factors (Z) and melting points (°C).

Parameter	Forastero Unroasted	Forastero Roasted	Criollo Unroasted	Criollo Roasted
a	7023.8	6466.8	5863.6	6544.0
b	15.608	14.538	13.191	14.374
R^2	0.994	0.994	0.996	0.988
E_a/kJ mol^{-1}	127.87	117.73	106.75	119.14
log Z	13.74	12.70	11.40	12.53
Z/min^{-1}	5.45×10^{13}	5.03×10^{12}	2.50×10^{11}	3.41×10^{12}
Melting point, T_1/°C	9.12 ± 0.01 ^{A}	15.90 ± 0.03 ^{B}	15.58 ± 0.16 ^{a}	15.92 ± 0.09 ^{a}
Melting point, T_2/°C	18.84 ± 0.06 ^{C}	20.66 ± 0.19 ^{C}	20.66 ± 0.01 ^{b}	19.67 ± 0.04 ^{b}

Values represent means ± standard deviations. Means with equal superscripts in each group for the same column are not significantly different ($p > 0.05$) by Tukey's test. The different lower-case letters (a–b) indicate significantly different values for Forastero beans ($p < 0.05$). The different capital letters (A–C) indicate significantly different values for Criollo beans ($p < 0.05$).

For the fat extracted from Criollo species, the values of activation energy reached 106.75 and 119.14 kJ mol^{-1}, for unroasted and roasted cocoa beans, respectively. For the fat extracted from Forastero species, the values of activation energy were significantly higher, 142.57 and 163.37 kJ mol^{-1}, for unroasted and roasted cocoa beans respectively. All the obtained values of activation energy are very high in comparison to other fats [18]. The thermal properties of fats are related to the fatty acid composition. The high values of E_a for cocoa butter can be due to the dominant contribution of saturated fatty acids. A similar correlation was observed by Khan et al. [39] who analysed edible oils with the use of UV-Vis and IR spectroscopy. The activation energy of the oxidation process for the fat from unroasted Criollo beans obtained in this article is comparable with the data in the literature [40]. However, the activation energy should not be used as the

only parameter to assess the oxidation stability of different lipid systems. The values of activation energy of oxidation presented in Table 4 for roasted and unroasted cocoa beans of two species agreed with previously confirmed stability of fat from roasted Criollo species over unroasted ones and unroasted Forastero species over roasted ones. In both Forastero cocoa beans unroasted and roasted, saturated fatty acids are predominantly esterified in sn-1, 3 TAGs positions but in roasted Forastero beans, we can find a lower amount of C16:0 and C18:0 in external positions of TAGs in comparison to unroasted Forastero beans where the amounts of these acids were higher in external positions. That distribution of saturated fatty acids can influence physical properties of cocoa butter which is connected with melting characteristics.

3.5. TGA/DTG Study of Cocoa Beans and Cocoa Butter Extracted from Unroasted and Roasted Cocoa Beans Criollo and Forastero

Thermogravimetry analysis (TGA) and the first derivative thermogravimetry (DTG) are excellent tools to examine the composition of mixtures, including food. From the TGA curve, the upper use temperature of a material, in the case of this study—fat—can be determined. Beyond this temperature, the material will begin to degrade. Hence, TGA can be applied complementary to DSC to estimate the thermal stability of the material. TGA and first derivative DTG were performed for ground cocoa beans of both species before and after roasting (Figures 3 and 4) as well as for fats extracted from these cocoa beans (Figures 5 and 6). The typical thermogravimetric behaviour of ground cocoa beans of Criollo (Peru) and Forastero (Ecuador) species showed significant diversity between the samples. Figure 3 shows TGA and DTG curves of unroasted and roasted cocoa beans Criollo in nitrogen (a, b) and oxygen (a', b'). For unroasted and roasted cocoa beans Criollo, two main mass losses were detected in the nitrogen atmosphere. The first mass change for unroasted cocoa beans Criollo of 20.39% was observed at temperature range 100–325 °C and for roasted cocoa beans of 19.89% at temperature range 75–300 °C. The second mass change for unroasted cocoa beans of 63.39% was observed at temperature range 325–500 °C and for roasted cocoa beans of 64.22% at temperature range 300–520 °C. Materazzi et al. [14] and Ostrowska-Ligęza et al. [24] studied the thermal properties of cocoa liquor. They stated that saccharides in the cocoa liquor were responsible for the first mass loss. The second significant mass loss was attributed to cocoa butter decomposition. The total mass loss for unroasted and roasted cocoa beans Criollo was not observed, the amount of the residues were 15.86 and 14.69%, respectively. This phenomenon can be explained by the high content of inorganic ingredients with high stability. The significant differences between unroasted and roasted cocoa beans have not been observed. Four stages of combustion on TGA and DTG curves for unroasted and roasted cocoa beans Criollo in oxygen were observed. First mass change for unroasted and roasted cocoa beans Criollo of 27.99 and 25.88%, respectively, was observed. In the case of unroasted and roasted cocoa beans Criollo, certain stages of mass changes took place in the following order: second mass loss of 35.23 and 37.68%, third mass change of 14.73 and 15.12% and fourth mass loss of 18.59 and 18.29%. All the mass changes were characterized by the same temperature ranges: first—150–275 °C; second—275–375 °C; third—375–450 °C and fourth—450–525 °C (Figure 3a',b'). According to Materazzi et al. [14] and Ostrowska-Ligęza et al. [24], cocoa liquor is a mixture of cocoa butter, cocoa powder, cocoa solid, antioxidant flavour and mineral compounds. They determined the thermal properties of dark chocolate ingredients in the air and oxygen atmosphere. In the case of the courses of DTG diagrams of unroasted and roasted cocoa beans Criollo the differences were observed. The DTG curve of roasted cocoa beans Criollo was characterized by a more distinct course than the DTG curve of unroasted cocoa beans Criollo. The roasting process causes the structure of cocoa beans has changed.

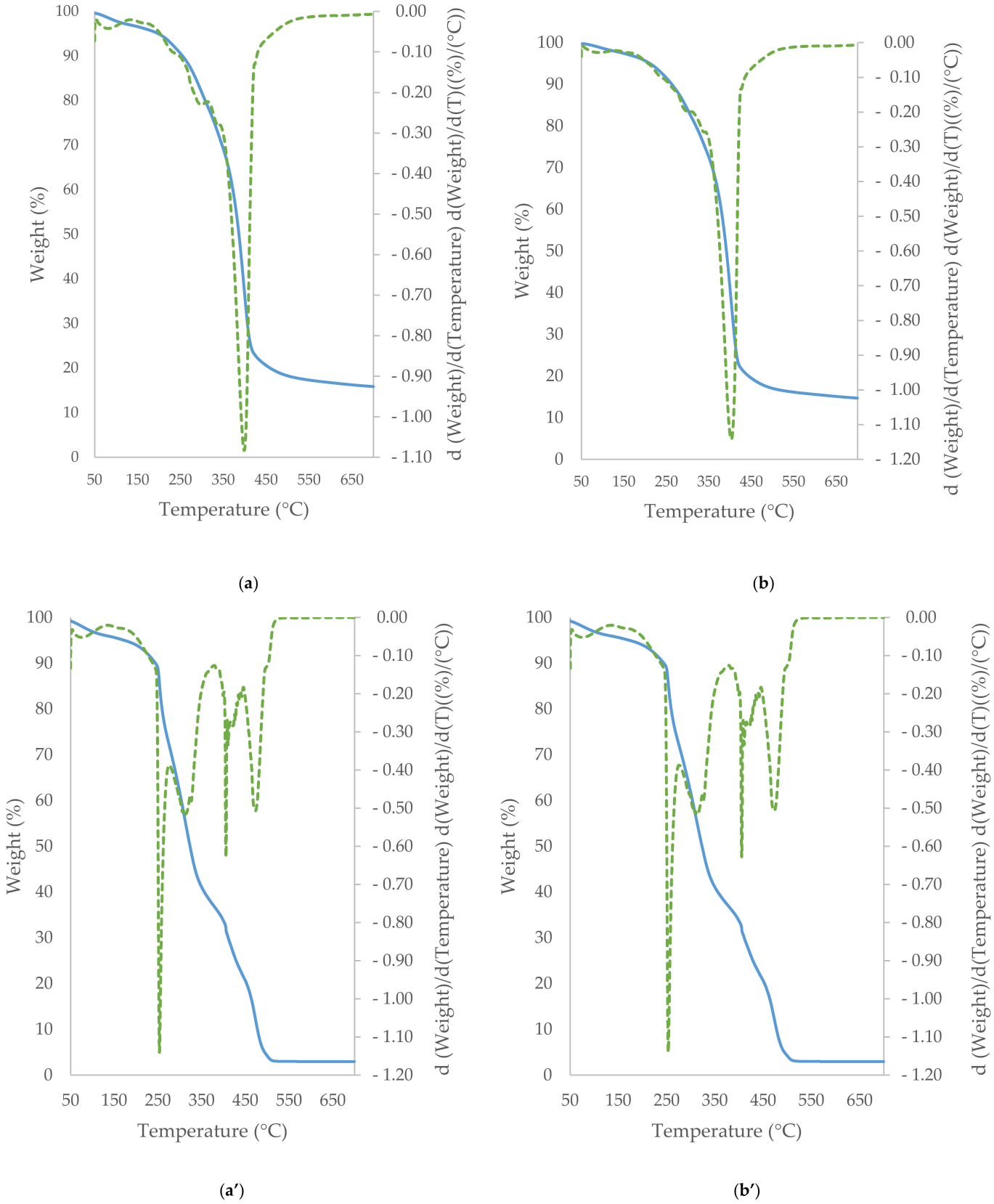

Figure 3. Thermogravimetry (TGA) and DTG curves of ground Criollo (Peru), unroasted (**a**) in nitrogen (**a′**) in oxygen and roasted (**b**) in nitrogen and (**b′**) in oxygen.

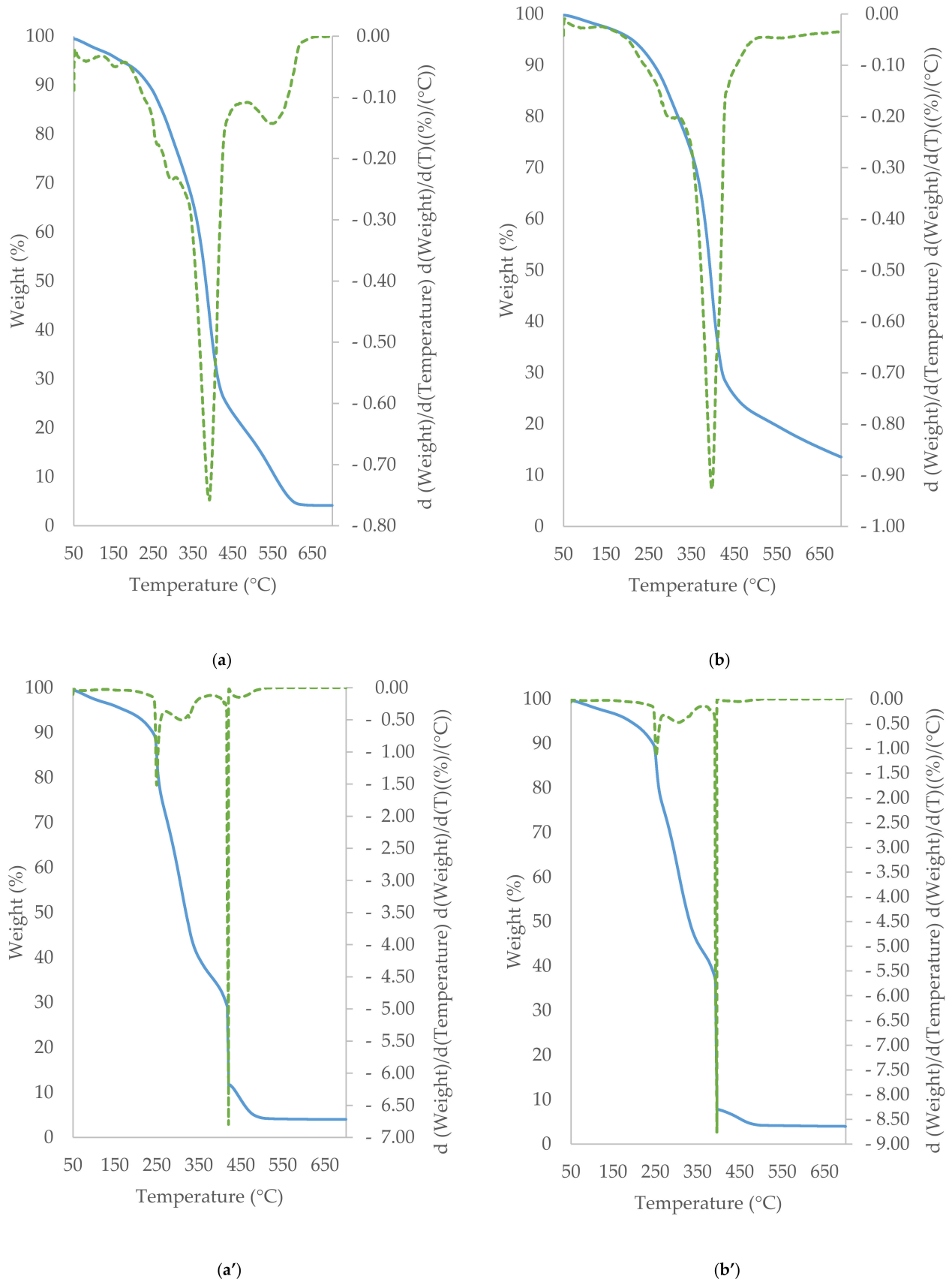

Figure 4. TGA and DTG curves of ground Forastero (Ecuador), unroasted (**a**) in nitrogen (**a′**) in oxygen and roasted (**b**) in nitrogen and (**b′**) in oxygen.

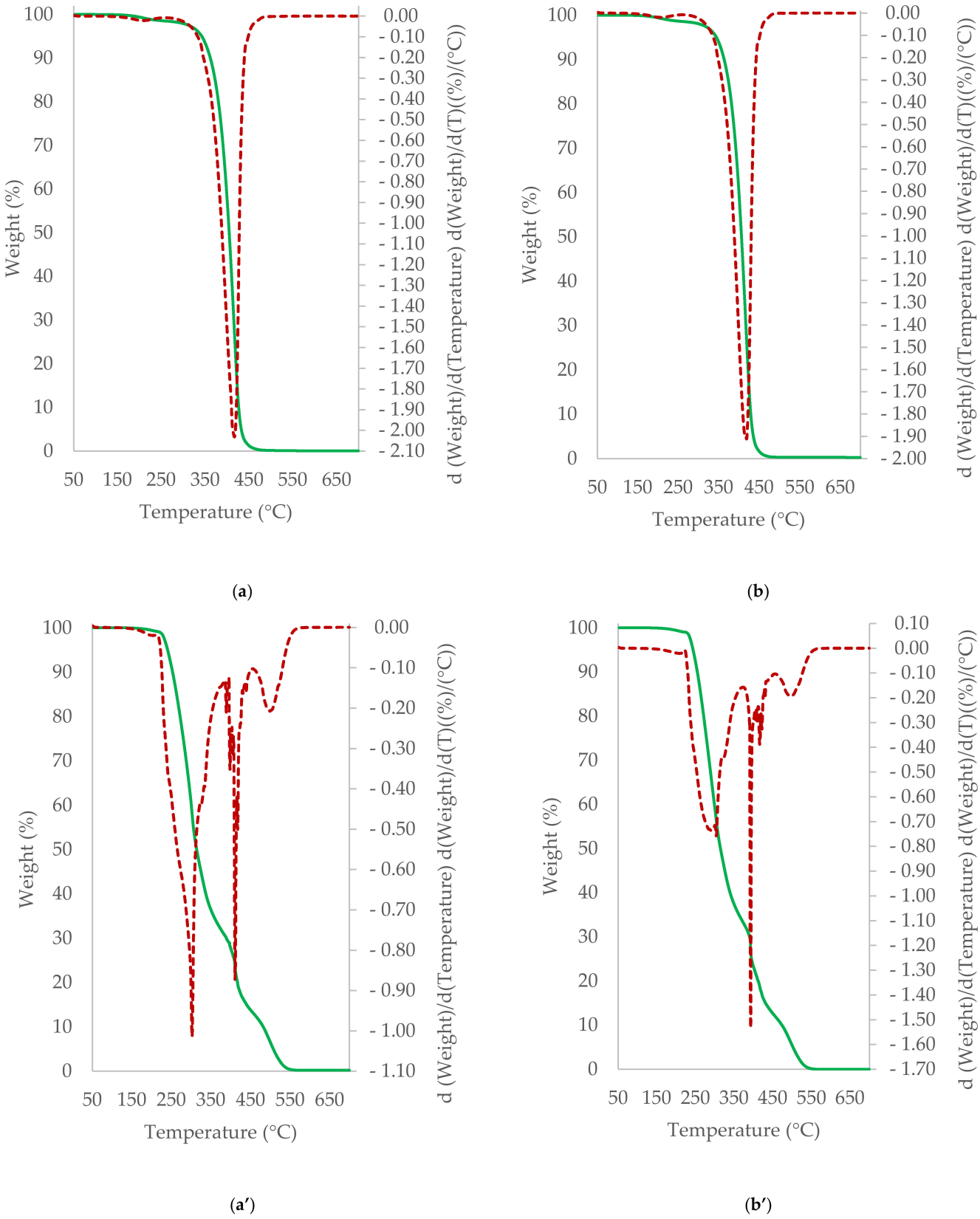

Figure 5. TGA and DTG curves of fats extracted from cocoa beans Criollo (Peru), unroasted (**a**) in nitrogen (**a′**) in oxygen and roasted (**b**) in nitrogen and (**b′**) in oxygen.

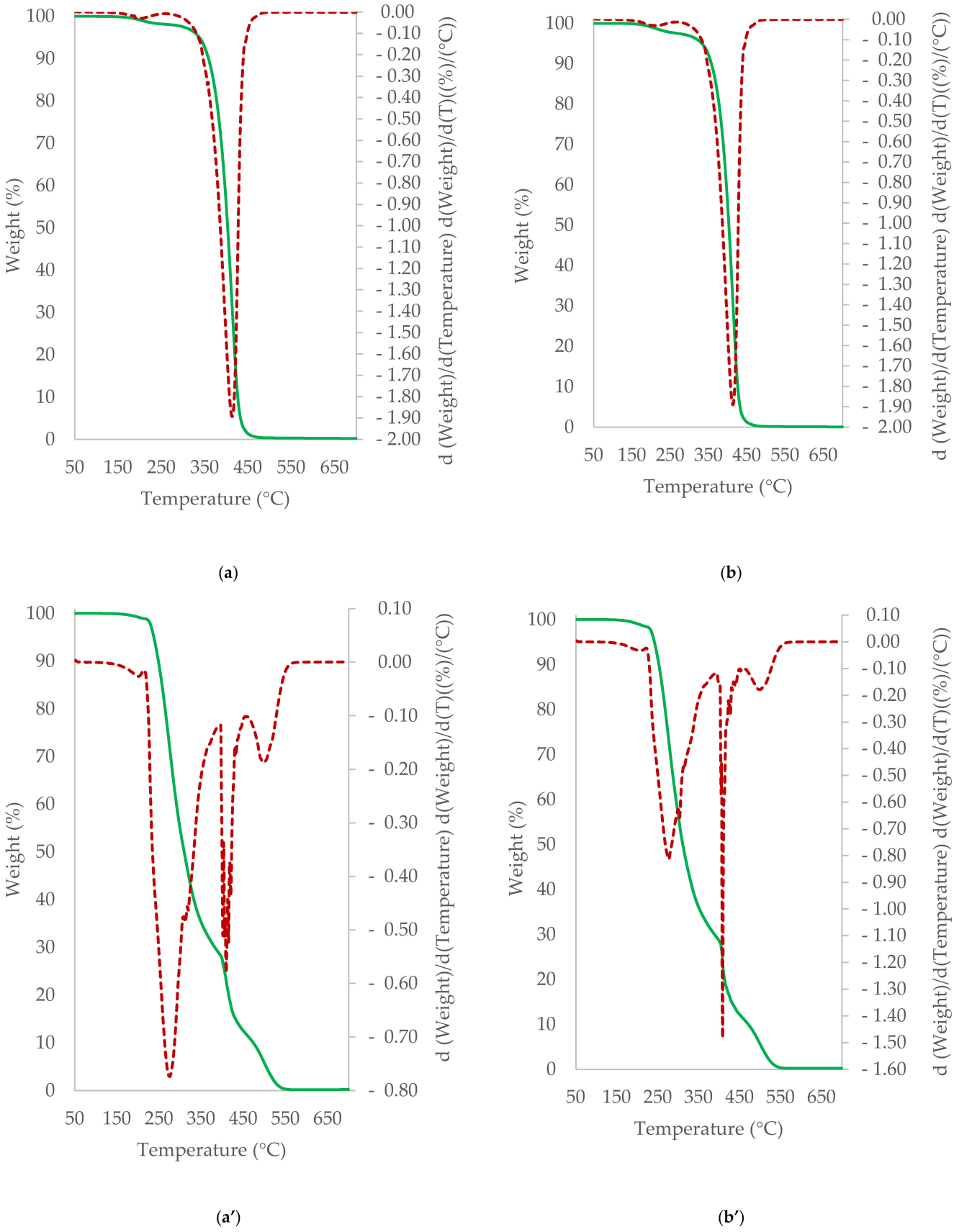

Figure 6. TGA and DTG curves of fats extracted from cocoa beans Forastero (Ecuador), unroasted (**a**) in nitrogen (**a′**) in oxygen and roasted (**b**) in nitrogen and (**b′**) in oxygen.

In Figure 4, the TGA and DTG curves of unroasted and roasted cocoa beans Forastero (Ecuador) in nitrogen (a, b) and oxygen (a', b') were presented. For unroasted and roasted cocoa beans Forastero, three main mass losses were detected in the nitrogen atmosphere. First mass loss of 21.89 and 20.59% at temperature range 120–325 °C for both (unroasted and roasted) cocoa beans Forastero was observed (Figure 4a,b). At this temperature decomposition of saccharides occurs. The saccharides are the components of cocoa beans Forastero and Criollo. The second mass loss of cocoa beans Forastero of 58.33 and 60.29% (unroasted and roasted) and the third mass change of 14.70 and 11.55% (unroasted and roasted) were at similar levels but the temperature range was different. The second mass loss for unroasted cocoa beans Forastero was observed in the temperature range from 325 to 475 °C, whereas in the case of roasted cocoa beans—325–525 °C. The maximum temperature of DTG curves for unroasted and roasted cocoa beans occurred at 390 and 397 °C, respectively. The third mass loss, at temperature range 475–625 °C (unroasted cocoa beans) and 525–625 °C (roasted cocoa beans) was an indication of inorganic residues in analysed cocoa beans. The courses of TGA and DTG curves indicated that cocoa bean samples of Criollo cocoa beans did not fully decompose. In Figure 4a',b', TGA/DTG diagrams for cocoa beans Forastero unroasted and roasted in oxygen were presented. Two main mass losses on TGA/DTG diagrams for unroasted and roasted cocoa beans Forastero were observed. The first and second mass loss indicated the decomposition of saccharides and oxidation of cocoa butter in the cocoa beans Forastero samples. The first stage ranged from 175 to 275 °C, second—from 275 to 450 °C and third from 450 to 700 °C (Figure 4a',b'). The maximum temperatures for unroasted Forastero cocoa beans were observed at 248 and 420 °C. The maximum peaks on DTG curves for roasted Forastero cocoa beans were obtained at 250 and 395 °C. The last mass loss finished with the total decomposition of cocoa beans in the oxygen atmosphere. The differences between the maximum temperature of second mass change stages for unroasted and roasted Forastero cocoa beans could have been caused by variation of distribution on sn-1 and sn-3 positions in triacyloglicerols of palmitic and stearic fatty acids (Table 2). Saturated fatty acids are characterized by the high temperature of combustion and long induction time of oxidation [41] which can be an explanation of better stability of cocoa butter.

Oxidation of lipids is affected by processing and storage conditions, the content of unsaturated fatty acids and their distribution in triacylglycerol molecule and the presence of antioxidants or prooxidants [42]. The thermogravimetric behaviours of the cocoa butter extracted from Criollo (Peru) and Forastero (Ecuador) species are shown in Figures 5 and 6 (TGA/DTG curves). The TGA/DTG curves of cocoa butter extracted from unroasted and roasted cocoa beans Criollo in nitrogen are characterized by one step of decomposition (Figure 5a,b). The maximum temperature on DTG curves for unroasted and roasted cocoa beans Criollo were obtained at 416 and 418 °C, respectively. The thermal decomposition of fat extracted from both types of Criollo cocoa beans was detected in the temperature range from 300 to 480 °C. There are no differences of values between mass loss for cocoa butter extracted from unroasted and roasted cocoa beans Criollo. The values of mass loss of 98.45 and 98.33% for unroasted and roasted cocoa beans Criollo, respectively, were obtained. Cocoa butter is characterized by polymorphic forms, but that phenomenon was not observed on TGA and DTG curves under nitrogen [43].

Cocoa butter extracted from unroasted and roasted cocoa beans Criollo TGA/DTG curves in oxygen atmosphere are characterized by different course. Three characteristic temperature ranges were observed: 220–400, 400–460 and 460–560 °C for fat extracted from unroasted cocoa beans Criollo (Figure 5a'). The first mass loss was the most significant and the value occurred at 72.03%. The other stage mass loss of 15.91% was associated and with the second temperature range. The last mass change was detected with a value of 11.95%. The first mass loss of 67.07% is observed at temperature range 225–375 °C for cocoa butter extracted from roasted cocoa beans Criollo (Figure 5b'). The second stage in thermal decomposition mass loss of 18.09% was associated with temperature range 375–440 °C. The third stage ranged from 440 to 560 °C and mass loss of level 14.77% was observed.

The maximum temperature for DTG curves of cocoa butter extracted from unroasted and roasted cocoa beans Criollo did not differ (Figure 5a',b'). According to de Souza et al. [44], the first stage on the DTG curve is correlated with the decomposition of polyunsaturated fatty acids, the second stage—decomposition of monounsaturated fatty acids and the third stage—decomposition of saturated fatty acids. Analysis de Souza et al. [44] consisted in determining thermal properties of sunflower oils with artificial antioxidants and without the addition of artificial antioxidants. Szabo et al. [45] determined thermal properties of sunflower oil and lard with the addition of natural antioxidants (obtained by alcoholic maceration of plants for example rosemary, basil and oregano). The results and conclusions of Szabo et al. [45] are similar to the results of de Souza et al. [44]. Górska et al. [46] studied the thermal properties of coffee silverskin and oil extracted from this material. The obtained results showed that coffee silverskin oil DTG curves indicated three stages of unsaturated, monounsaturated and saturated fatty acids thermal decomposition. The study of de Souza et al. [44], Szabo et al. [45] and Górska et al. [46] confirmed the results obtained in the research of cocoa butter extracted from unroasted and roasted cocoa beans Criollo thermal properties.

The distinction between the thermal characteristics of various vegetable oils is due, mainly to differences in the distribution of fatty acids in the sample. Thus, the complexity of the thermal profiles of vegetable oils can vary due to the principal constitutes [47]. Established on the results from Table 2, it can be concluded that unsaturated fatty acids were located mainly in position sn-2 of triacylglycerols (share of oleic acid was from 64.66 to 82.73% and share of linoleic acid was from 59.78 to 90.05%). The internal position composed mainly of unsaturated fatty acid may have an influence on the rate of the oxidation process of this fatty acid. The external positions in triacylglycerols are occupied by saturated fatty acids (share of palmitic acid was from 3.37 to 23.71% and share of stearic acid was from 2.78 to 22.21%). This distribution of fatty acids in triacylglycerols may have an influence on the arrangement of molecules in three-dimensional structures. The distribution of these fatty acids affects the melting profiles and crystallization, and thus the course of the DTG curves [40]. The results in Table 2 do not sufficiently explain the differences in the course of the TGA/DTG curves. Cocoa butter is characterized by six polymorphic forms [48]. Different polymorphic forms have different physical properties which are typical for cocoa butter.

The differences in the course of DTG curves of cocoa butter extracted from roasted cocoa beans Criollo can be explained by polymorphic phenomena. The TGA/DTG curves of fat extracted from unroasted and roasted cocoa beans Forastero in nitrogen were characterized by a similar course to cocoa butter extracted from beans Criollo (Figure 6a,b). The thermal decomposition of cocoa butter extracted from unroasted and roasted cocoa beans Forastero in nitrogen occurred in one stage ranged from 250 to 470 °C and was accompanied by mass loss of 98.91% for unroasted beans and 98.02% for roasted ones. The profile of TGA/DTG curves shows three stages of thermal decomposition both for cocoa butter extracted from unroasted and roasted cocoa beans Forastero as can be observed in Figure 6a',b'. TGA/DTG diagrams were characterized by the same temperature ranges for three stages of thermal decomposition: first—from 220 to 400 °C, second—from 400 to 460 °C and third—from 460 to 560 °C. The first stages occurred for cocoa butter extracted from unroasted and roasted cocoa beans Forastero with corresponding mass losses of 71.66% and 70.87%, respectively. The second and third stages were accompanied by much smaller mass losses for both, unroasted and roasted Forastero cocoa beans, 16.37% and 17.48% with respect to the second thermal event and 11.79% and 11.39% with respect to the third stage of decomposition, respectively (Figure 6a',b'). Changes in weight loss were observed during the thermal decomposition of cocoa butter extracted from unroasted and roasted Forastero cocoa beans. The stages of thermal decomposition, courses and shapes TGA/DTG diagrams of cocoa butter extracted from unroasted and roasted cocoa beans of Criollo and Forastero type showed features of similarities.

4. Conclusions

Four different techniques were applied to compare the properties of roasted and unroasted cocoa beans of two different species. Different values of iron content in unroasted and roasted cocoa beans Criollo and Forastero indicate various technics of roasting beans. The results of DSC were related to TGA and GC and unobvious patterns were found. The kinetic information was helpful to assess the oxidative stability of cocoa butter. The cocoa butter extracted from unroasted Forastero from Ecuador was characterized by the highest values of activation energy E_a. The roasting process has no influence on the composition and distribution of fatty acids in cocoa butter. The melting characteristics of cocoa butter extracted from roasted Criollo species were comparable to its unroasted version. The same trend was not observed for Forastero species. TGA and DTG were revealed to be useful tools for the analysis of cocoa beans and the fats extracted from these cocoa beans. The results showed the possibility to distinguish fats derived from different species of cocoa tree to control the quality of the final product, e.g., chocolate. The Criollo cocoa bean is of the best quality and the harvest of these beans reaches about 3% of the world's harvest. It makes this species very expensive. Forastero cocoa beans are more popular and cheaper, making them more often used in food production. The use of certain beans and processing conditions depends on which particular product is to be manufactured.

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