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Vegan and sugar-substituted chocolates: assessing physicochemical characteristics by NMR relaxometry, rheology, and DSC

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Abstract

The main physicochemical characteristics of novel artisanal chocolates (both dark and milky) intended for vegan consumers or for those requiring assumption of fewer simple sugars, were analysed. Replacement of milk (with coconut copra, almonds, and soy protein isolates), and sucrose (with coconut sugars, stevia and erythritol, respectively) in dark chocolate, were accounted for by means of texture analysis, rheology, water activity, fatty acid composition, differential scanning calorimetry (DSC) and fast field cycling (FFC) nuclear magnetic resonance (NMR) relaxometry. The vegan sample (i.e., the milk-less one) showed lower values of hardness and adhesiveness as well as a larger peak in the melting behavior at the calorimetric evaluation (DSC). Moreover, the absence of milk resulted in the halving of the yield stress and a decrease in both the apparent and Casson's viscosity. In the sample of chocolate with less sucrose, the peak temperatures measured at the DSC indicate crystallization of cocoa butter in its best form ($V\beta_2$), unlike in dark chocolate, due to the different sugar composition. Similarly, the Casson yield stress (τ_0), increased significantly (almost 70%), with the substitution of sugar. Finally, the results of NMR FFC relaxometry made it possible to identify aggregates of different sizes, laying the basis for its use as a rapid, non-destructive method for chocolate analysis.

Keywords Vegan chocolate · Low-sucrose chocolate · DSC · Rheology · NMR relaxometry · Texture

Introduction

Chocolate is valued more for its sensory characteristics and polyphenol content than for its nutritional properties. For this reason, confectionery companies, to broaden the consumer audience, are looking for new chocolate formulations that can satisfy dietary styles associated with cultural choices or intolerances and pathologies. Chocolate contains about 30% sucrose or even more. This is needed not only to favor sweetness, but also to increase volume and mass, as well as to ameliorate chocolate texture, aroma and mouthfeel

[1]. Consumers increasingly demand a reduction in simple sugar content in food, including diabetics who must limit or avoid the consumption of mono- and disaccharides. Stevia is intensely sweet natural compound, approximately 300 times as sweet as sucrose on weight basis [1]. Erythritol, is a viable alternative for replacing sugars in chocolate, since its sweetness is 60% that of sucrose, with a glycemic response and energy value of 0 and 0.2 kcal g^{-1} respectively, compared to 100 and 4 kcal g^{-1} of sucrose: due to its lower absorption in the body [2, 3]. However, a very recent study revealed that erythritol could be associated with the risk of major adverse cardiovascular events [4], although, to this day, the main food safety agencies (i.e., FDA and EFSA) have not yet ruled on the matter. Less used is, instead, coconut sugar, a natural sweetener obtained by evaporation of the sap of *Cocos nucifera*, is less unhealthy than sucrose, due to the presence of minerals and vitamins, proteins, phenols, and low glycaemic index [5]. Moreover, lactose-intolerant or even vegan subjects are unable to consume milk-based chocolates due to the presence of powdered cow milk which is added during chocolate production. As a general remark, addition of milk allows production of sweeter chocolate with more

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malleability and thermolability than dark chocolate [6–8]. It is therefore desirable to study new formulations for producing both milk-free chocolates with vegetable substitutes and dark chocolate with sweeteners and few added simple sugars (hereafter referred to as sugar substitutes), to increase the number of consumers. The structure of chocolate, a very important parameter for the consumer's perception of its quality, is correlated with the forms of crystallization resulting from the cocoa butter components used during chocolate preparation. The crystalline arrangement of the (V) form crystals in the cocoa butter allows a melting point between 33 and 34 °C [9]. This means that a well-structured chocolate can be stored at room temperature for a sufficiently long time, thereby making easier logistics operations without the use of refrigerated containers. Chocolate flow properties of chocolate are other important parameters for evaluating its structure. Moreover, the taste of chocolate in the mouth is directly influenced by viscosity. Therefore, chocolate bad taste can be perceived when wrong flow/viscosity characteristics are achieved [10, 11]. In addition, final chocolate texture, appearance and flavour can be considered as key attributes for consumer choice and acceptability [12–14]. All the keys are related to the phase transitions of polymorphic forms in fat systems by their melting points which, in turn, are monitored by differential scanning calorimeter (DSC) [15]. Chocolate characteristics can be associated also with the molecular dynamics of the complex mixtures making this food product. Molecular dynamics can be explored by low field NMR relaxometry. This is a fast, reproducible, accurate and non-invasive technique, which can be applied to expand knowledge of chocolate stabilization characteristics [16, 17]. The aim of this study was to evaluate the main chemical-physical properties of novel vegan and substituted-sugar chocolates. Therefore, two different chocolates were handcrafted. In milk-less products, viable for lactose-intolerant and vegan people, milk powder was replaced with dried coconut copra, roasted almonds, and isolated soy proteins. In the case of substituted-sugar dark chocolate, sucrose was replaced with stevia, erythritol and coconut sugar. Comparison with traditional products (milk- and sucrose-containing chocolates) was carried out by analyzing water activity, fatty acids content, texture, melting point by differential scanning calorimetry (DSC), rheology and fast field cycling (FFC) NMR relaxometry.

Materials and methods

Materials

The chocolate samples were made in an artisan confectionery laboratory (Pasticceria Cappello, Palermo, Italy) using, for both the control and the experimental samples, a refiner with counter-rotating porphyry rollers for mixing (Ing. Polin EC. S.p.A., Verona, Italy) and obtaining the paste of cocoa, and a bench robot set at 60 °C for 4 h of conching. The chocolate obtained was manually tempered on marble and molded into circular shapes in silicone molds with a weight of 7–8 g, 4.5 mm of thickness and 35 mm of diameter for each shape obtained; cooled for 10 min at 10 °C then left at room temperature (18–20 °C).

Sample coding is as follows:

- MiC: milk-containing chocolate
- VeC: milk-less vegan chocolate
- DaC: sucrose-containing dark chocolate
- SuSDC: substituted-sugar dark chocolate

Raw materials

Cocoa mass was purchased by Valhrona (France), Manjari Pur Madagascar 100%, for milk chocolate samples production, and Araguani Pur Venezuela 100% cocoa mass for dark chocolate, were used. The cocoa butter and whole milk powder were purchased from ICAM Professional SPA, (Lecco, Italy). Dehydrated coconut (Pearls of Samarkand, Sri Lanka), powdered isolated soy proteins (Natural Soy Isolate, ProLabs, Eros, EuroSup), pure stevia powder (UOP Durante, Italy), and crystal coconut sugar (Monte Nativo, Sri Lanka), were purchased on online marketplace. Almonds were purchased by Musumeci company, (Bronte, CT, Italy). Natural Bourbon vanilla powdered by Vanilla Gourmet (Pescara, Italy); soy lecithin by Nutrition&Santè (Lecinova, Italy); by Chimpex (Caivano, NA, Italy). The percentage composition of the ingredients in the different samples is shown in Table 1. The formulation of the SuSDC sample results in an about 8% reduction in calorific value: with about 2333.4 kJ 100 g^{-1}, compared to 2531.7 kJ g^{-1} in

Table 1 Formulations in milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Ingredient %	MiC	VeC	DaC	SuSDC
Cocoa derivatives	41.5	44.8	74.5	77.0
Sucrose	34.5	37.0	25.0	/
Milk powder	23.5	/	/	/
Almond	/	7.7	/	/
Coconut copra	/	5.0	/	/
Isolated soy proteins	/	5.0	/	/
Erythritol	/	/	/	9.9
Stevia	/	/	/	0.1
Coconut sugar	/	/	/	12.5*
Soy lechitin	0.5	0.5	0.5	0.5

*approximately 6% sucrose

DaC sample. All samples produced and the analyses below were performed in triplicate.

Water activity and dry matter

The analysis of the water activity was carried out with the HygroPalm-23 instrument (Rotronic, Basserdorf, Germany) following the ISO 21807:2004 method. The samples were all analysed in triplicate. Percentage of dry matter was analysed by using official analytical method (AOAC 930.15, 1990). Samples were dried in oven at 105 °C for 3 h, then weight loss was measured.

Fatty acids groups

Fatty acids were identified and quantified, after methylation [18], by using a gas chromatograph (HRGC 5300; Carlo Erba, Milano, Italy) fitted with a 30 m × 0.25 mm, 0.20 μm i.d. Rtx-2330 (Restek, Bellefonte, PA) capillary column and a FID detector.

Texture analysis

Penetration test was made using a TaXT2 texture analyzer (Stable Microsystem) equipped with a cylindrical probe of 5 mm diameter (P5). The area of the probe was ten times smaller than the area of the sample. The hardness of the sample was the maximum force registered during the test; the adhesiveness was the negative force recorded during the probe withdrawal from the sample after the penetration. The operative parameters used for the test were as follows: pre-test speed 2 mm s^{-1}; test speed 2 mm s^{-1}; post-test speed 5 mm s^{-1}; distance 50%; load cell 25 kg. The test was conducted at 18 ± 0.5 °C.

Differential scanning calorimetry

The melting profiles of chocolate samples were determined using Differential Scanning Calorimetry (DSC) (Q Series DSC, TA Instruments, New Castle, Delaware, USA). 4–5 mg of samples were loaded in an aluminium pan and nitrogen was used as transport inert gas at a flow rate of 50 ml min^{-1}. An empty pan was used as a reference, while indium was used for instrument calibration. Samples were initially equilibrated at 20 °C and then heated to 60 °C at 5 °C min^{-1} rate. Thermograms were analysed by TA Universal Analysis software (TA Instruments, New Castle, Delaware, USA), defining the onset temperature (Tonset), melting peak temperature (Tm), end temperature (Tend) and the melting enthalpy (ΔHm). Melting index (T$_{index}$) was calculated as (T$_{end}$ -T$_{onset}$) and measures the melting range [19].

Rheological analysis

The rheological properties of chocolate were studied using a Haake Mars III rheometer (Thermo Scientific) equipped with Couette geometry (with coaxial cylinders) with an outer cylinder diameter of 43 mm, the inner one of 41 mm, and a vertical distance (from the bottom) between the two cylinders equal to 3 mm. Chocolate flow parameters were determined by following the official method viscosity (ICA, 2000) of cocoa and chocolate products (Analytical method 46. CAOBISCO, Brussels). Chocolate was placed in a closed glass container. It was heated in a benchtop oven at 52 °C for at least 1 h before evaluating the flow properties at 40 °C. In detail, the test was programmed in 4 steps: 1) pre-shear at a fixed shear rate ($\dot{\gamma}$) of 5 s^{-1} for 5 min; 2) ascending ramp with ($\dot{\gamma}$) from 2 to 50 s^{-1} in 3 min, 3) fixed shear rate at ($\dot{\gamma}$) = 50 s^{-1} for 1 min; 4) descending ramp with ($\dot{\gamma}$) from 50 to 2 s^{-1} in 3 min.

The data recorded in the upward flow curve section were then interpolated using Casson's model:

$$\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta_c \dot{\gamma}} \tag{1}$$

where τ_0 is the Casson yield stress, i.e., the stress required for the fluid to start flowing, and η_c is the Casson viscosity indicating the force required to maintain chocolate flow during the test. The apparent viscosity, measured at 40 s^{-1}, relates to the viscosity of the chocolate at high shear rates and represents the behavior of the chocolate during pumping and mixing processes. Apparent viscosity values were taken from the ascending curve [20]. Thixotropy was measured by the hysteresis area formed by the difference between the upward and downward curves. The larger the hysteresis area, the longer the time needed for the fluid to recover its structure.

Fast-field-cycling (FFC) NMR relaxometry

Details about the technique have been already reported elsewhere [21]. Here, only a brief report on the used experimental conditions is reported. All the experiments were conducted on a Stelar Smartracer Fast-Field-Cycling Relaxometer (Stelar s.r.l., Mede, PV–Italy) set at a constant temperature of 25 °C. The proton spins were polarized at a polarization field (B$_{POL}$) corresponding to a proton Larmor frequency (ν_L) of 10 MHz for a period (T$_{POL}$) of about five times the T$_1$ estimated at this frequency. After each B$_{POL}$, the magnetic field intensity (indicated as B$_{RLX}$) was systematically changed in the proton Larmor frequency ν_L comprised in the range 0.01–10 MHz. The period τ, during which B$_{RLX}$ was applied, has been varied on 32 logarithmic spaced time sets, each of them adjusted at every relaxation

field to optimize the sampling of the decay/recovery curves. Free induction decays were recorded following a single ^{1}H 90° pulse applied at an acquisition field (B_{ACQ}) corresponding to ν_L of 7.2 MHz. A time domain of 100 µs sampled with 512 points was applied. Field-switching time was 3 ms, while spectrometer dead time was 15 µs. For all the experiments, a recycle delay of 2 s was used. A non-polarized FFC sequence was applied when the relaxation magnetic fields were in the range of the proton Larmor frequencies comprised between 20 and 10 MHz. A polarized FFC sequence was applied for B_{RLX} values ranging between 3 and 0.01 MHz [21].

All the decay/recovery curves acquired by applying the aforementioned experimental runs were exported to OriginPro 7.5 SR6 (Version 7.5885, OriginLab Corporation, Northampton, MA, USA) to apply the stretched exponential function (also known as Kohlraush–Williams–Watts function) reported in Eq. (1) [22].

$$I(\tau) = I_0 exp\left[-\left(\frac{\tau}{T_1}\right)^k\right] + y_0 \qquad (2)$$

Here, $I(\tau)$ is the magnetization intensity at a given τ value; I_0 is the magnetization intensity at the asymptote of the decay/recovery curve; τ is the period of time during which B_{RLX} is applied; T_1 is the longitudinal relaxation time; k is a parameter accounting for the relaxometry complexity of the samples; y_0 is the experimental offset. Equation (1) accounts for the large sample heterogeneity resulting in a multiexponential behavior of the decay/recovery curves. In particular, this equation can be considered as a superposition of exponential contributions, which describes the likely physical picture of some distributions in T_1. Its application has the advantage that it can handle a wide range of relaxometry behaviors within only one single model. For this reason, any assumption about the number of exponentials to use for modelling the FFC NMR relaxometry data is not necessary [21].

The NMRD profiles (i.e., R1 = 1/T_1–vs–ν_L curves) were modelled according to the free-model analysis [23] to obtain the distribution of correlation times from which information about the dynamic domains in chocolates were obtained.

Statistical analysis

The means and standard deviations of experimental results were calculated from three replicate trials. To determine their differences, one-way ANOVA and Tukey's test were used. Differences at $p < 0.05$ were considered significant and were indicated with different letters. All statistical analyses were done via the SPSS Version 20.0 statistic software package (SPSS Inc., Chicago, IL, USA).

Table 2 Percentage of saturated fatty acids (SFA), mono unsatured fatty acids (MUFA) and poly unsatured fatty acids (PUFA) in milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Samples	SFA (%)	MUFA (%)	PUFA(%)
MiC	60.5 ± 0.7[a]	35.4 ± 0.4[b]	4.00 ± 0.03[b]
VeC	66.3 ± 0.9[b]	29.9 ± 0.4[a]	3.80 ± 0.02[b]
DaC	62.4 ± 0.6[a]	34.3 ± 0.5[b]	3.30 ± 0.02[a]
SuSDC	64.2 ± 0.8[a]	32.1 ± 0.3[b]	3.60 ± 0.01[a]

*Different letters mean significant differences ($p < 0.05$)

Table 3 Water activity and dry matter values expressed in milk chocolate control (MiC), vegan (VeC), dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Samples	aw (mean ± st.dv)	Dry Matter (%)
MiC	0.44 ± 0.01[a]	98.4 ± 0.8[a]
VeC	0.439 ± 0.008[a]	98.4 ± 0.7[a]
DaC	0.375 ± 0.002[b]	98.4 ± 0.7[a]
SuSDC	0.378 ± 0.009[b]	97.0 ± 0.9[a]

*Different letters mean significant differences ($p < 0.05$)

Results and Discussion

Fatty acids groups

Table 1 reports the ingredients used to produce the different artisanal chocolates. All the fatty acid groups (i.e., the saturated fatty acids, SFA, the mono- and polyunsaturated fatty acids, MUFA, and PUFA, respectively) significantly varied between VeC and MiC samples (Table 2). This difference can be explained by considering the different formulation (Table 1) and the chemical composition of the newly added vegetable ingredients. As expected, the DaC and SuSDC samples revealed similar contents of the three fatty acid groups (Table 2). Fat crystallization in chocolate can be understood by studying the molecular interactions between different species of saturated and unsaturated fatty acids, which are differently bound to glycerol carbons. Such analysis is beyond the scope of this research; however, the fatty acid data will be discussed to interpret some of the following results.

Water activity and dry matter

Water activity (aw) refers to the water capacity to be bound to different components in a food matrix. In particular, the lower the aw value, the more bound are the water molecules. In this study, MiC and VeC samples showed similar

aw values (Table 3), thereby revealing that replacement of milk with milk-substitutes does not affect water binding. The same results were obtained for DaC and SuSDC. In fact, as for VeC and MiC, DaC and SuSDC showed the same aw values. However, it must be noticed that the water activities for MiC-VeC were higher than those measured for DaC- SuSDC. This can be explained by considering that milk powder (in MiC) and coconut sugar (in VeC) contain highly hygroscopic saccharides such as inulin in coconut [24, 25]. The latter, indeed, lacks in both DaC and SuSDC. Finally, all the samples (i.e., Mic-Vec, and DaC-SuSDC) revealed the same dry matter content. This is very important because in the paragraphs below the FFC NMR relaxometry results are discussed. All the differences are not due to aw and dry matter values, but to the chemical nature of the organic chocolate components.

## Differential Scanning Calorimetry

The main quality parameters of chocolate, which are good mouth meltability, snapping properties at room temperature, and glossiness, depend on the crystallization form of cocoa butter used as fat phase. In particular, the (V) is the preferred polymorph for commercial chocolate production, because it guarantees the proper reaching of quality parameters cited above. DSC is widely used for the evaluation of fat polymorphism in processed food products and for the evaluation of extent of fat crystal network organization during the chocolate manufacturing. Figure 1, 2, 3 shows the melting profiles registered during the heating of four chocolate samples. Each thermogram has been evaluated measuring the onset peak corresponds to the temperature at which a specific crystalline form begins to melt, the maximum peak corresponding to the temperature at which the melting curve reaches its peak maximum, the end of melting and the enthalpy related to the whole melting peak (Table 4). The DaC and SuSDC samples show a single peak, more sharply defined in the SuSDC chocolate, in contrast to MiC and VeC, for which a double
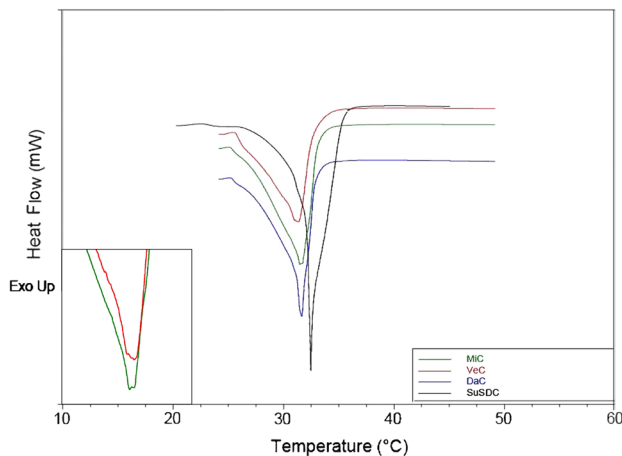
Fig. 1 Differential Scanning Calorimetry (DSC) plot of: **a** milk chocolate control (MiC); **b** vegan chocolate (VeC); **c** dark chocolate (DaC), and **d** sugar-substituted chocolate (SuSDC) samples
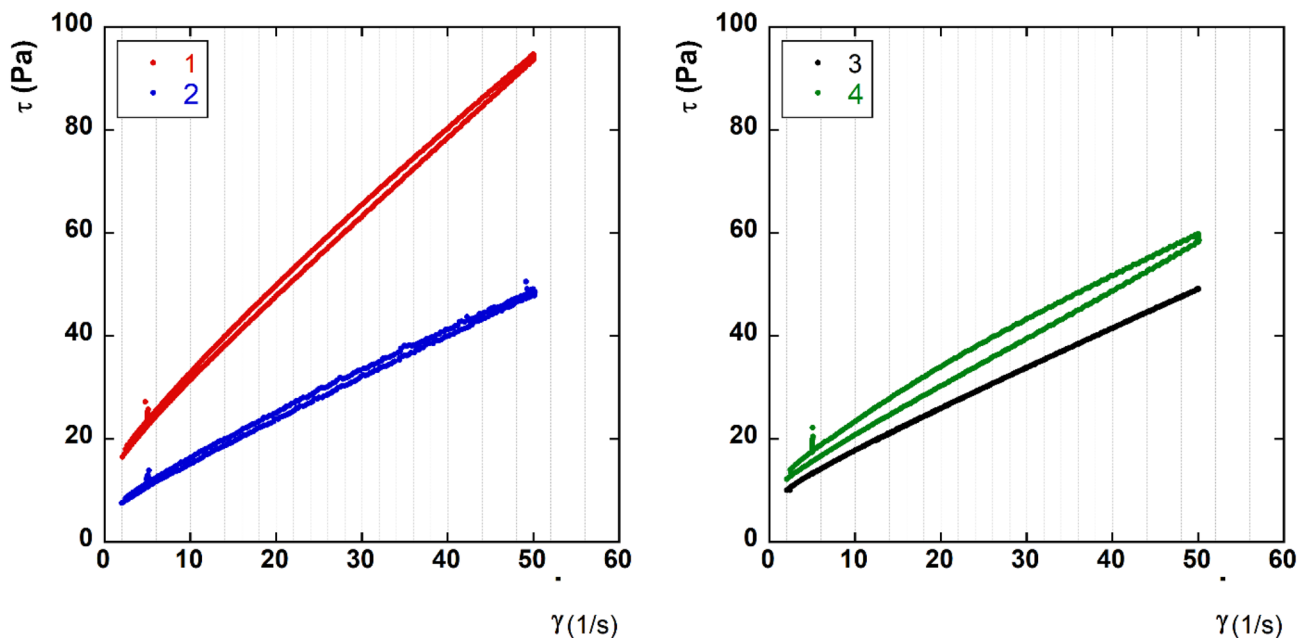
Fig. 2 Left: flow curves of milk chocolate MiC (1) and vegan chocolate VeC (2). Right: flow curves of dark chocolate DaC (3), and SuSDC dark chocolate (4)
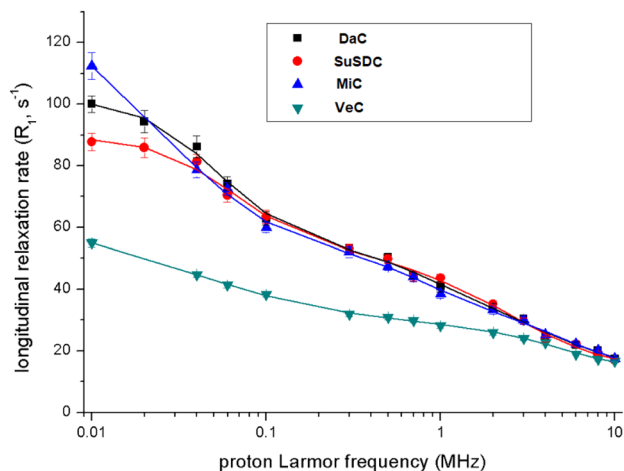
Fig. 3 NMR Relaxometry evaluating relaxation rate for milk chocolate (MiC); vegan chocolate (VeC); dark chocolate control (DaC), and sugar-substituted dark chocolate (SuSDC)

melting transition is visible (inset of Fig. 4). The melting temperatures of cocoa butter polymorphs are reported as follows: I sub-α (17.3 °C), II α (23.3 °C), III β'$_2$ (25.5 °C), IV β'$_1$ (27.5 °C), V β$_2$ (33.8 °C), VI β$_1$ (36.3 °C) [26]. The maximum peak temperatures detected for chocolate samples, suggest the crystallization of cocoa butter in its (V β$_2$) form, the best, only in SuSDC sample. The observed differences among the melting profiles (Fig. 1) and the registered temperatures (Table 4) can be ascribed by the various ingredients employed for the formulation of chocolates. The double melting transition in Mic and VeC sample could be caused by the triglyceride's composition of milk and coconut copra fat, that affect crystallization behavior of cocoa butter CB [27]. Moreover, the differences between melting peaks as well as the enthalpy values in DaC and SuSDC samples can be due to the different sugar composition, too. In fact, sugars and their particle sizes affect the CB crystallization because they represent nuclei which provide the "seed" around which fat crystals grow [28, 29]. The melting enthalpy and T index for DaC chocolate was significantly higher than those of SuSDC, ($p < 0.05$) indicating a higher extent of fat crystallization

in systems, probability due to the simultaneous presence of high amount of CB and homogeneous sugar phase in systems.

Texture analysis

Penetration test was used to determine hardness of the chocolate disks and adhesiveness, defined as the work required to pull the sample away from a surface [30–32]. Hardness of chocolate is a good parameter that points out proper control of temperature and stability of the fat crystal network formed during tempering process [33, 34]. Treated samples (VeC and SuSDC) showed hardness values lower to the control samples (MiC and DaC), respectively (Table 5). These data suggested that samples with lower melting enthalpy tend to have a softer structure at the same temperature, *i.e.,* when subjected to the stress of texture analysis, showing a lower resistance to probe penetration [35]. DSC results also showed a lower Tpeak for VeC sample, related to the lower point of fusion. Moreover, VeC sample had the lowest value of adhesiveness. Hardness value was highest in DaC, because of the absence of any other substances that can affect the typical structure of the dark chocolate. SuSDC had the lower hardness value than (DaC) sample, owing to the presence of coconut sugar, highly hygroscopic sweetener containing a significant amount of inulin (about 5 g 100 g^{-1}). These data confirmed that the substitution of sucrose by other sugars provides low hardness values compared to reference chocolate [10].

Rheology

Chocolate can be considered as a suspension of solid particles in a Newtonian fluid (cocoa butter). It is well known that chocolate does not usually have Newtonian behavior, which was also confirmed for the samples analysed, all of which exhibited the characteristics of Casson's plastic fluid. Chocolate rheology is usually quantified using parameters like yield stress (τ_0) and apparent viscosity (measured at shear rate 40 s^{-1}). Yield stress is a material property and is the stress corresponding to the yield point at which the material begins to deform plastically [36]. Furthermore,

Table 4 Melting properties by Differential Scanning Calorimetry (DSC) in milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Sample	T_{onset} (°C)	$T_{peak\ 1}$ (°C)	$T_{peak\ 2}$ (°C)	T_{end} (°C)	Tindex (°C)	ΔH_m [J/g]
MiC	25.3 ± 0.2^a	31.5 ± 0.4^a	31.7 ± 0.4^a	34.6 ± 0.1^a	9.3 ± 0.3	40 ± 9^a
VeC	25.3 ± 0.4^a	31.0 ± 0.1^a	31.3 ± 0.1^a	34.6 ± 0.5^a	9.3 ± 0.9	35 ± 11^a
DaC	25.2 ± 0.3^a	31.60 ± 0.09^b	/	35.7 ± 0.9^a	$10. \pm 1$	55 ± 9^b
SuSDC	32.0 ± 0.1^b	32.5 ± 0.4^b	/	35.8 ± 0.2^a	3.8 ± 0.3	33.10 ± 0.02^a

*Different letters mean significant differences ($p < 0.05$)
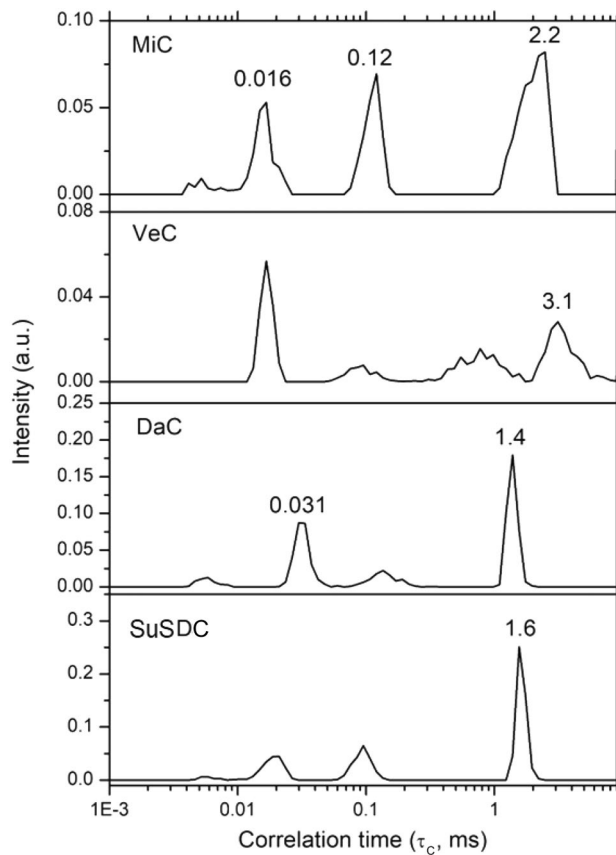
Fig. 4 Correlation times obtained by inverse transformation of data resulted by relaxation rate (Fig. 3) for milk chocolate (MiC); vegan chocolate (VeC); dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Table 5 Texture parameters evaluating height, hardness and adhesiveness in milk chocolate (MiC), vegan (VeC), dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Samples	Height (mm)	Hardness (g)	Adhesiveness (g s)
MiC	5.2 ± 0.3^a	$(12.5 \pm 0.5) \times 10^{3b}$	-0.8 ± 0.8^b
VeC	4.5 ± 0.4^a	$(7.8 \pm 0.3) \times 10^{3a}$	-18 ± 9^a
DaC	5.4 ± 0.4^a	$(21.7 \pm 0.3) \times 10^{3c}$	-2 ± 1^b
SuSDC	4.5 ± 0.5^a	$(9.7 \pm 1.5) \times 10^{3a}$	-0.4 ± 0.4^b

*Different letters mean significant differences ($p < 0.05$)

water contents may have a negative effect on flow properties resulting in higher yield value and viscosity. Values of apparent viscosity reported in Table 6 showed that the vegan chocolate had a lower viscosity at high shear rate compared to the parent milk product and that the sugar replacing in dark chocolate resulted in a slower increase of η app compared to the control. Results of flow curves data fitting to the Casson equation and the measurement of the hysteresis area (thixotropy) are shown in Table 6 [20]. For all the samples, the

Table 6 Rheological properties in milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC), and sugar-substituted dark chocolate (SuSDC)

Samples	η_{app} (Pa s) shear rate 40/s	τ_0 (Pa)	η_c (Pa s)	Thixotropy (Pa/s)
MiC	2.00 ± 0.02^d	6.55 ± 0.03^d	1.020 ± 0.002^d	335 ± 15.4^b
VeC	1.02 ± 0.01^a	2.87 ± 0.02^a	0.560 ± 0.001^c	524 ± 25.2^d
DaC	1.04 ± 0.01^b	3.82 ± 0.04^b	0.500 ± 0.002^a	106 ± 14.1^a
SuSDC	1.29 ± 0.02^c	6.45 ± 0.03^c	0.540 ± 0.001^b	445 ± 22.0^c

*Different letters mean significant differences ($p < 0.05$)

shear rate applied resulted in a non-linear response in terms of shear stress (Fig. 2) indicating that chocolate aggregates aligned to the flow as the shear rate increase thus opposing less resistance, which is the reason why chocolate samples showed shear thinning behavior. As for τ_0 values, the new formulations showed an opposite trend. In fact, in vegan chocolate, the substitution of milk powder caused more than a halving of the yield stress and a similar decrease in apparent and Casson viscosity. These data, consistent with the texture value, showed a variation in the rheological properties of vegan chocolate compared to traditional one. The differences between dark and sugar-substituted chocolate showed an inverse trend in τ_0 values, which increased significantly (almost 70%) with sugar substitution, while the increase in viscosity was smaller (about 9%). This last was consistent other results [37], in which it was reported that dark sweetened with isomalt presented higher Casson viscosity than that of the sucrose-containing chocolate, confirming that the chocolate formulations comprising high levels of sugar substitutes had higher apparent and Casson viscosity, and yield stress than the those of the control [38]. Thixotropy is a function of time-dependent fluid and can be evaluated through apparent viscosity or shear stress decreasing with the time of shear at constant rate. Thixotropy is calculated from the area of loop or a specific point on the ramp curves of shear stress or apparent viscosity at a specific shear rate [39]. Values reported in Table 6 were calculated as the area of loop formed between the upward and downward flow curves and as can be observed all samples showed thixotropic behavior. However, thixotropy indicates the role of time in the process of re-structuring after shearing.

FAST-FIELD-CYCLING (FFC) NMR relaxometry

In Fig. 3, Nuclear Magnetic Resonance Dispersion (NMRD) profiles, reporting the longitudinal relaxation rate as a function of the Larmor frequency of the applied electromagnetic field, is shown. VeC sample (the vegan chocolate) with fat replacers of milk powder, shows the lowest relaxation rate as compared to the other samples. This is justified by the

different molecular motion affecting the fluctuation of the local electromagnetic field. A faster molecular motion is related to longer relaxation times T_1 and slower relaxation rate values [40]. In this case, it is possible to suggest that the different fat composition that characterizes VeC sample, affects the interaction of molecules of cocoa butter that serve for the crystallization process. This is because of the interaction between cocoa butter and milk powder replacers' fats generating aggregates with higher molecular mobility as compared to the other chocolate samples. Nothing else can be qualitatively deduced by the visual inspection of Fig. 3. For this reason, the model described elsewhere [23] has been applied.

The inverse transformation of the function related to the relaxometry NMRD showed a time domain graph where different correlation times, each referred to the dynamic components of the chocolate matrixes, are reported (Fig. 4). The longest correlation times can be associated to larger aggregates moving slowly. Conversely, as correlation time values decrease, molecular dynamics increases because of the reduction of the molecular aggregates. Therefore, starting from the left-hand side of Fig. 4, it is possible to state that the first proton population (at around 0.016 ms for MiC, VeC, and SuSDC, respectively, while at 0.031 ms for DaC) is due to the presence of substances with higher proton mobility and faster correlation time. According to the preparation procedure of the different chocolates, the band at the shortest τ_C value can be associated to the presence of lactose and sucrose, that is two small-sized disaccharides. Moreover, the second correlation time at around 0.12 ms is conceivably due to proteins. In particular, in MiC, a net peak related to proteins is visible. This is explained by considering that this type of chocolate contains the largest number of proteins due to the use of cow milk, whereas the other products are made by cow milk surrogate proteins which enable a lower and broader band centered at 0.12 ms. Finally, the correlation time at the longest values (2.2 ms for MiC, 3.1 ms for VeC, 1.4, and 1.6 ms for DaC and SuSDC, respectively) is related to non-polar substances such as the lipid fraction. The correlation time for this component is the longest in all the graphs, thereby corresponding to a high interaction of this component in the matrix. In fact, the cocoa butter is the main component of the continuous phase and responsible of the crystalline state of chocolate. MiC showed a high and large peak because of presence of both cocoa butter and milk powder fats. Both are mainly composed by saturated fatty acids that make them solid with the lowest proton mobility.

According to the molecular mobility within the chocolate, the first peak of VeC sample (the one with milk powder replacers) is related to sucrose with lowest correlation time. A shorter and less net peak is related to the presence of isolated soybean proteins that were added in the mixture to replace the amount of milk powder proteins. Then, the range of signals related to lipids present in the VeC mixture was more variegated, with short and jagged population of peaks, and it was not possible to distinguish each peak related to a single component. But it was possible to identify solid fat components (related to cocoa butter and all the saturated fatty acids of coconut copra) with higher correlation times, while liquid fat components (oils, mainly unsaturated and polyunsaturated fatty acids from almond and coconut) have lower correlation times compared to solid fats, because of the slightly higher mobility of their protons compared to the solid fat components [17]. This different composition of the fat content in the sample is possibly the main reason of the interference of the different lipids in the crystallization process of cocoa butter. Results of NMR analysis are comparable with DSC results, where MiC and VeC sample showed a lower Tpeak of melting (compared to the dark chocolate samples) and a wider peak during the transition phase of chocolates. Sucrose is present also in the third sample (DaC), but at a lower concentration (25% of added sucrose). The corresponding band is slightly shifted to the right, compared to the first two samples. The explanation can be found in the Fig. 3, where the DaC sample shows higher Longitudinal Relaxation Rate compared to SuSDC dark chocolate sample. Higher relaxation rates and lower relaxation times are related to a deeper solid–solid or sugar–solid interaction in food [41]. That means that sucrose may be more embedded within the cocoa butter continuous phase compared to the other chocolate samples. The proton population related to proteins in this sample is related to the proteins of cocoa mass, generally bound within tannins complexes, but visible in DaC and SuSDC. The last net peak in the DaC graph is referred to cocoa butter, the highest peak with the highest correlation time, because of the high level of aggregation that is responsible for the crystallinity of chocolate. Alternative sugars as sucrose replacers were used for the formulation of the fourth sample (SuSDC). One of these was the coconut sugar (a low-absorbable sugar containing sucrose, and inulin, that is a β-D-fructose polymer), then, erythritol and stevia sweetener. Figure 4 reveals a sucrose band that is less intense than that present in the other samples. This is in line with the sucrose concentration (about 50%) in coconut sugar [41], so SuSDC contains about 6.0% of sucrose (Table 1). That is, about one quarter of the DaC sample, so it is visible as a very small peak in the band of molecules with faster molecular movement at the beginning of Fig. 4 in SuSDC. The relaxation rate of the SuSDC sample shown in Fig. 3 is lower than that of DaC, due to less interaction between the alternative sugars within the cocoa butter network. This is consistent with the sugar-solid interactions elsewhere explained [42]. The cocoa mass protein was included in the last peak in both the DaC and SuSDC samples (Fig. 3).

Conclusion

The substitution of milk powder with vegetable ingredients showed several chemical-physical differences with milk chocolate. In particular, the mixture of coconut copra, almonds and soy protein isolates, added instead of milk powder, affected the consistency, with lower hardness and adhesiveness values, and the melting behavior at DSC, with a smaller enthalpy peak. Partial substitution of sucrose with other sugars in dark chocolate changed the texture due to the presence of a hygroscopic ingredient (such as inulin in coconut sugar), which reduced the hardness values in the sugar-substituted sample. Substitution of sucrose by sweeteners, on the other hand, showed that the crystallization of cocoa butter was closer to the best form (V β_2) in the new chocolate, while the Casson yield stress (τ_0) increased significantly (almost 70%) with sugar substitution. Finally, NMR FFC relaxometry proved to be a promising tool for the rapid and non-destructive assessment of chocolate quality. In fact, the application of the free-model analysis provides useful chocolate fingerprints that can be used to recognize in only one shot the origin and nature of different chocolates, provided that a large number of samples is analyzed. Therefore, more analyses are needed to better address FFC NMR investigations.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This study does not contain any studies with human participants or animals performed by any of the authors.

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