Novel vegan and sugar-substituted chocolates. Part I: physical-chemical characterization

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Abstract

The confectionery industry is increasingly adopting new solutions and possible formulations to expand the ranges of chocolate products that support food styles linked to either cultural or health choices. The chemical-physical characteristics of chocolates (dark and milk) produced with traditional formulations or intended for vegan or demanding less simple sugars consumers (with a 10% reduction in calorific value), were analysed. The effects of the substitution of milk with coconut copra, almond and isolated soy proteins, and the replacement of sucrose with coconut sugars, stevia and erythritol, have been accounted for by analysing texture, rheology and water activity, differential scanning calorimetry (DSC) and fast field cycling (FFC) nuclear magnetic resonance (NMR) relaxometry. The plant-based sample showed lower values for hardness and adhesiveness in the texture analysis, and a larger peak in the melting behaviour at the DSC. Moreover, the substitution of milk powder caused more than a halving of the yield stress and a similar decrease in apparent and Casson viscosity. The crystallisation of cocoa butter in the substituted-sugar sample involved the $\beta$ V form, the most desirable crystal form in high-quality chocolate. Results by FFC NMR relaxometry allowed identification of differently sized aggregates whose chemical nature is discussed. FFC NMR relaxometry data confirm those by rheological and DSC investigations.

Introduction

Sweets farms are looking for new chocolate formulations that can meet dietary styles associated to either cultural choices or intolerances, and pathologies. Increasingly consumers demand less simple sugars in food, including diabetics who must limit or avoid the consumption of mono- and disaccharides. Stevia, as well as erythritol, is a viable alternative for replacing sugars in chocolate, thus making this product more appetible for diabetics [1, 2]. Less used is, instead, coconut sugar, a natural sweetener obtained by evaporation of the sap of *Cocos nucifera* [3]. 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 malleability and thermolability than dark chocolate [4, 5]. Noticeably, attention must be paid in obtaining chocolate products that must have sensory characteristics like milk- and sugar-containing systems. Chocolate structure is directly related to the size of the tiny particles and crystals deriving from the components of the cocoa butter used during chocolate preparation. The crystalline arrangement of the $\beta$ form crystals (V) in the cocoa butter allows a melting point between 33 and 34°C [6]. Chocolate flow properties are important for assessing 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 [7, 8]. In addition, final chocolate texture, appearance and flavour can be considered as key attributes for consumer choice and acceptability [9–11]. All the aforementioned 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) [12]. Chocolate characteristics can be associated also with the molecular dynamics of the complex mixtures making this food product that 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 stabilisation characteristics [13, 14]. The aim of this study was to evaluate the main chemical-physical properties of novel plant-based and substituted-sugar chocolates. Two different chocolates have been prepared. In milk-less products 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 analysing water activity, fatty acids content, texture, melting point by differential scanning calorimetry (DSC), rheology and fast field cycling NMR relaxometry.

Materials and Methods

Sampling

The chocolate samples were made in an artisan confectionery laboratory (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 hours of conching. The chocolate obtained was manually tempered on marble and molded into circular shapes in silicone molds with a weight of 7-8g, 4.5 mm of thickness and 35 mm of diameter for each shape obtained. Flow chart of production process is reported in Fig. 1.

Sample coding is as follows:

**MiC:** Milk-containing Chocolate **VeC:** milk-less Vegan Chocolate

**DaC:** sucrose-containing Dark Chocolate **SuSC:** Substituted-Sugar Chocolate

Raw materials

The cocoa mass was purchased from Valhrona (France): respectively Manjari Pur Madagascar 100%, for milk chocolate, and Araguani Pur Venezuela 100% for dark chocolate. The cocoa butter was 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); erythritol by Chimpex (Caivano, NA, Italy). The percentage composition of the ingredients in the different samples is shown in Table 1. The formulation of the SuSC sample results in a 10% reduction in calorific value: about 424.58 kCal 100 g⁻¹, compared to 472.67 kCal 100 g⁻¹ in DaC sample.
Table 1
Formulations of milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC) and sugar-substituted dark chocolate (SuSC).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>MiC</th>
<th>VeC</th>
<th>DaC</th>
<th>SuSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa Mass</td>
<td>41</td>
<td>44.6</td>
<td>75</td>
<td>77</td>
</tr>
<tr>
<td>Sucrose</td>
<td>34.5</td>
<td>37</td>
<td>24</td>
<td>/</td>
</tr>
<tr>
<td>Milk Powder</td>
<td>23.5</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Almond</td>
<td>/</td>
<td>7.5</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Coconut Copra</td>
<td>/</td>
<td>5</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Isolated soy proteins</td>
<td>/</td>
<td>5</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Erithritol</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>9.6</td>
</tr>
<tr>
<td>Stevia</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>0.1</td>
</tr>
<tr>
<td>Coconut Sugar</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>12.1</td>
</tr>
<tr>
<td>Soy lechitin</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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. Dry matter was analysed by using official analytical method (AOAC 930.15, 1990).

Texture analysis

Texture analysis was performed by using a TaXT2 texture analyser (Stable Microsystem) equipped with a cylindrical probe (P35) with which the chewing test with double compression was simulated. The 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 (Q Series DSC, TA Instruments, New Castle, Delaware, USA). 5–10 mg of chocolate 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 melting peak temperature (Tm) and the melting enthalpy (ΔHm).
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 gap of 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 hour before evaluating the flow properties at 40°C. 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:

\[
\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta_c \dot{\gamma}}
\]

where \(\tau_0\) is the Casson yield stress, i.e., the stress required for the fluid to start flowing, and \(\eta_c\) is the Casson viscosity indicating the force required to maintain chocolate flow during the test. 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 [15]. 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 (\(n_L\)) 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 \(n_L\) comprised in the range 0.01–10 MHz. The period \(\tau\), 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 \(n_L\) 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 (Conte, 2021). 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) in order to
apply the stretched exponential function (also known as Kohlraush–Williams–Watts function) reported in Eq. (2) [16].

\[ I(\tau) = I_0 e^{-(\frac{\tau}{T_1})^k} + y_0 \]

2

Here, \( I(\tau) \) is the magnetization intensity at a given \( \tau \) value; \( I_0 \) is the magnetization intensity at the asymptote of the decay/recovery curve; \( \tau \) 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. Eq. (1) accounts for the large sample heterogeneity resulting in a multiexponential behaviour 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 is able to 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 [15]. The NMRD profiles (i.e., \( R_1 = 1/T_1 \)-vs-\( n_L \) curves) were modelled according to the free-model analysis elsewhere reported [17], to obtain the distribution of correlation times from which information about the dynamic domains in chocolates were obtained.

**Results and Discussion**

**Water activity and dry matter**

The MiC and VeC samples showed higher values of water activity than the other two samples (Table 2). This can be related to the presence of milk powder in (MiC) and coconut sugar in (VeC), a highly hygroscopic saccharide containing inulin [18, 19], whose solubility, wettability and dispersibility have already been studied [20].
<table>
<thead>
<tr>
<th>Samples</th>
<th>Aw (mean ± st.dv)</th>
<th>Dry Matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiC</td>
<td>0.445 ± 0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.44 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VeC</td>
<td>0.439 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.36 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DaC</td>
<td>0.375 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.38 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SuSC</td>
<td>0.378 ± 0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.99 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters mean significant differences (p < 0.05).

**Differential Scanning Calorimetry**

The main quality parameters of chocolate, which are good mouth meltability, snapping properties, and glossiness, depend on the crystallization form of cocoa butter (CB) used as fat phase. Differential scanning calorimetry (DSC) is widely used for the evaluation of fat polymorphism and crystal network organization in chocolate (Fig. 1). 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 3). DaC and SuSC samples show a single peak, and in the case of SuSC a sharp one, with a maximum peak in contrast to MiC and VeC, for which it possible noting the presence of a double melting transition (inset of Fig. 1). The melting temperature of the cocoa butter for the polymorphic forms are I (17.3°C), II (23.3°C), III (25.5°C), IV (27.5°C), V<sub>β</sub> (33.8°C) and VI (36.3°C) [6]. The maximum peak temperature detected for the chocolate samples shows that the crystallisation of cocoa butter in the SuSC sample is the β V form, the most desirable crystal form in high-quality chocolate. The double melting transition in MiC and VeC sample could be caused by the triglyceride’s composition of milk and coconut copra fat that according with other authors [21], affect crystallization behaviour of CB.

Differences between melting peaks as well as the enthalpy values of DaC and SuSC samples can be due to the different sugar composition. Sugars and their particle sizes also affect the CB crystallization because they represent nuclei which provide the “seed” around which fat crystals grow [22]. The melting enthalpy in DaC chocolate was significantly higher than those of MiC, VeC and SuSC (p < 0.05) indicating a higher extent of fat crystallization in systems probability due to the simultaneous presence of high amount of CB (75%) and homogeneous sugar phase in systems [23].
Table 3
Melting properties by Differential Scanning Calorimetry (DSC) in milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC) and sugar-substituted dark chocolate (SuSC).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{onset}$ (°C)</th>
<th>$T_{peak 1}$ (°C)</th>
<th>$T_{peak 2}$ (°C)</th>
<th>$T_{end}$ (°C)</th>
<th>$T_{index}$ (°C)</th>
<th>$\Delta H_m$ [J/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiC</td>
<td>25.3±0.2$^a$</td>
<td>31.47±0.41$^a$</td>
<td>31.67±0.42$^a$</td>
<td>34.6±0.1$^a$</td>
<td>9.33</td>
<td>39.7±8.66$^a$</td>
</tr>
<tr>
<td>VeC</td>
<td>25.3±0.4$^a$</td>
<td>30.97±0.10$^a$</td>
<td>31.31±0.11$^a$</td>
<td>34.6±0.5$^a$</td>
<td>9.32</td>
<td>35.4±11.24$^a$</td>
</tr>
<tr>
<td>DaC</td>
<td>25.2±0.3$^b$</td>
<td>31.64±0.09$^b$</td>
<td>/</td>
<td>35.7±0.9$^a$</td>
<td>10.45</td>
<td>54.9±8.66$^b$</td>
</tr>
<tr>
<td>SuSC</td>
<td>32.0 ± 0.1$^b$</td>
<td>32.47 ± 0.41$^b$</td>
<td>/</td>
<td>35.8 ± 0.2$^a$</td>
<td>3.82</td>
<td>33.1 ± 0.02$^a$</td>
</tr>
</tbody>
</table>

Different letters mean significant differences (p < 0.05).

Texture Analysis

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 [24, 25]. All treatments (VeC and SuSC) showed hardness values lower compared to the control samples (MiC and DaC) (Table 4). These data confirmed that samples with lower melting points tend to have a softer structure at the same temperature [26], i.e., when subjected to the stress of texture analysis, showing a lower resistance to probe penetration. DSC results also showed a lower $T_{peak}$ for VeC sample, related to the lower point of fusion. VeC sample also had the lowest value of adhesiveness. Hardness value was highest in DaC, because of the absence of any other substances that can affect the structure of the chocolate. SuSC 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 100g$^{-1}$) [19]. Data confirmed that sucrose replacement with high ratios of sugar substitutes provide low hardness values respect to the controls [27].

Table 4
Texture parameters in milk chocolate (MiC), vegan chocolate (VeC), dark chocolate (DaC) and sugar-substituted dark chocolate (SuSC).

<table>
<thead>
<tr>
<th>Samples</th>
<th>height (mm)</th>
<th>hardness (g)</th>
<th>adhesiveness (g s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiC</td>
<td>5.18 ± 0.30</td>
<td>12534 ± 530</td>
<td>-0.82 ± 0.76</td>
</tr>
<tr>
<td>VeC</td>
<td>4.50 ± 0.36</td>
<td>7836 ± 303</td>
<td>-17.9 ± 8.84</td>
</tr>
<tr>
<td>DaC</td>
<td>5.43 ± 0.40</td>
<td>21760 ± 323</td>
<td>-2.22 ± 1.36</td>
</tr>
<tr>
<td>SuSC</td>
<td>4.47 ± 0.48</td>
<td>9688 ± 1521</td>
<td>-0.35 ± 0.36</td>
</tr>
</tbody>
</table>

Rheology

It is well known that chocolate does not usually have Newtonian behaviour, 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 ($\tau_0$) and apparent (plastic) viscosity (measured at shear rate 5 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 [7]. Results of flow curves data fitting to the Casson equation and the measurement of the hysteresis area (thixotropy) are shown in Table 5. For all the samples, the 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 behaviour. As for $\tau_0$ 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 $\tau_0$ values, which increased significantly (almost 70%) with sugar substitution, while the increase in viscosity was smaller (about 9%). This last was consistent with other results [28], in which 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 [29]. 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 [30]. Values reported in Table 5 were calculated as the area of loop formed between the upward and downward flow curves and as can be observed all samples showed thixotropic behaviour. Considering the thixotropic data [31], it appears that the VeC and SuSC samples have a more complex structure than the related reference chocolates (MiC and DaC).

<table>
<thead>
<tr>
<th>Samples</th>
<th>$\eta_{app}$ (Pa s)</th>
<th>$\tau_0$ (Pa)</th>
<th>$\eta_c$ (Pa s)</th>
<th>Thixotropy (Pa/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiC</td>
<td>23.8</td>
<td>6.55 ± 0.03</td>
<td>1.02 ± 0.002</td>
<td>335.3</td>
</tr>
<tr>
<td>VeC</td>
<td>11.5</td>
<td>2.87 ± 0.02</td>
<td>0.56 ± 0.001</td>
<td>524.8</td>
</tr>
<tr>
<td>DaC</td>
<td>13.2</td>
<td>3.82 ± 0.04</td>
<td>0.50 ± 0.002</td>
<td>106.6</td>
</tr>
<tr>
<td>SuSC</td>
<td>17.6</td>
<td>6.45 ± 0.03</td>
<td>0.54 ± 0.001</td>
<td>445.5</td>
</tr>
</tbody>
</table>

**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 [32]. 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 [17] 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 SuSC, 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 $t_C$ 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 content 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 SuSC, 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 [14]. 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 (24% of 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 SuSC dark chocolate sample. Higher relaxation rates and lower relaxation times are related to a deeper solid-solid or sugar-solid interaction in food [33]. 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 SuSC. 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 (SuSC). One of these was the coconut sugar (a low-absorbable sugar containing sucrose, and inulin, a β-D-fructose polymer [34], 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 [35], so SuSC contains about 6.0% of the total amount of sucrose (Table 1). That is, about one third 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 SuSC. The relaxation rate of the SuSC 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 [33]. The cocoa mass protein was included in the last peak in both the DaC and SuSC samples (Fig. 4).

**Conclusions**

The substitution of milk powder with vegetable ingredients showed strong differences with milk chocolate. The mixture of coconut copra, almonds and soy protein isolate, added in place of milk powder, affected the texture, with lower hardness and adhesiveness values, and the melting behaviour at DSC, with a wider peak. Moreover, vegan chocolate had the lowest longitudinal relaxation rate obtained by NMR, the lowest apparent viscosity and Casson yield stress, and the highest thixotropy value among all samples. In dark chocolate, the texture was partially influenced by the presence of a hygroscopic ingredient (inulin in coconut sugar), which reduced the hardness values in the substituted-sugar sample. Substitution of sucrose with sweeteners, on the other hand, showed that the crystallisation of cocoa butter was closer to the best form (β V) in the novel sample, while no other changes were recorded by rheology and NMR relaxometry analysis.

**Declarations**

**Ethical Approval**

“not applicable”
Competing interests

“I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper”.

Authors’ contributions

OC; FC; CL; PLM: Investigation, Formal analysis. PC & PLM: Data curation, Supervision, Writing - review & editing. DA: Methodology, Investigation, Writing - review & editing. LC & FT Conceptualization, Methodology, Investigation, Validation; Review & editing, Supervision)

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References


Figures

Figure 1

Differential Scanning Calorimetry (DSC) plot of a) milk chocolate (MiC); b) vegan chocolate (VeC); c) dark chocolate (DaC) and d) sugar-substituted chocolate (SuSC).
Figure 2

Left: flow curves of milk chocolate MiC (1) and vegan chocolate VeC (2). Right: flow curves of dark chocolate DaC (3) and SuSC dark chocolate (4).
Figure 3

NMR Relaxometry evaluating Relaxation Rate for milk chocolate (MiC); vegan chocolate (VeC); dark chocolate control (DaC) and sugar-substituted dark chocolate (SuSC).
Figure 4

Correlation times obtained by inverse transformation of data resulted by Relaxation Rate (Figure 3) for milk chocolate (MiC); vegan chocolate (VeC); dark chocolate (DaC) and sugar-substituted dark chocolate (SuSC).