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1 Microstructural and rheological characteristics of dark, milk and white chocolate: a
2 comparative study

3

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12

13 **Abstract**

14 Three different chocolate types: dark, milk and white were characterized and compared for the
15 microstructural and rheological (fundamental and empirical) characteristics. A light microscope
16 coupled to an image analysis system was employed in order to evaluate the particle size, the
17 network structure and the distance between particles of each matrix. Rheological parameters (yield
18 stress, viscosity, thixotropy) were evaluated by using a stress–strain rheometer, while mechanical
19 properties (consistency and cohesiveness) were analyzed using a texture analyzer. The *Power law*,
20 *Casson* and *Windhab* rheological models were used in order to better explain the rheological
21 behaviour of chocolate samples.

22 Results showed that white chocolate, with the highest amount of fat in formulation, had the smallest
23 sized particles, the less aggregate structure and the lowest yield stress, viscosity and thixotropic
24 values. Dark chocolate samples instead presented the highest aggregate structure, with less void
25 spaces between particles that involved the highest rheological parameter values. Milk chocolate
26 matrix exhibited intermediate microstructural and rheological characteristics compared to dark and
27 white chocolate ones.

28

29 **Keywords:** chocolate, microstructure, rheology, modelling

30

31 1. Introduction

32 Chocolate is a dispersion of around 70% of fine particles such as cocoa powder, sugar and milk
33 solids in a continuous phase made up of fats, generally cocoa butter and milk fat, depending on the
34 specific formulation (Fernandez et al., 2013). There are three main types of chocolate: dark, milk
35 and white, with notable differences between them (Awad and Marangoni, 2006; Afoakwa et al.,
36 2008; Beckett, 2010). Dark chocolate formulation consists mainly of cocoa liquor, sugar and cocoa
37 butter. Milk chocolate is made up of sugar, cocoa butter, milk solids and cocoa liquor; white
38 chocolate is made up of sugar, cocoa butter and milk solids (Rousseau, 2007). Chocolate can also
39 include emulsifiers such as lecithin and polyglycerol polyricinoleate (PGPR) as well as salt,
40 flavourings or spices. The directives 2000/36/EC, relating to cocoa and chocolate products intended
41 for human consumption define “Dark chocolate”, as the product obtained from cocoa products and
42 sugars, containing not less than 35 % total dry cocoa solids, including not less than 18 % cocoa
43 butter and not less than 14 % of dry non-fat cocoa solids. “Milk chocolate” is defined as the product
44 obtained from cocoa products, sugar and milk or milk products, with not less than 25 % total dry
45 cocoa solids; not less than 14 % dry milk solids (obtained by partly or totally dehydrated full cream
46 milk, semi- or full-skimmed milk, cream, or from partly or completely dehydrated cream, butter or
47 milk fat); not less than 2.5 % dry non-fat cocoa solids; not less than 3.5 % milk fat and not less than
48 25 % total fat (cocoa butter and milk fat). By the same directive “White chocolate” is defined as the
49 product obtained from cocoa butter, milk or milk products and sugar, containing not less than 20 %
50 cocoa butter and not less than 14 % dry milk solids (obtained by partly or totally dehydrated full
51 cream milk; semi- or full-skimmed milk; cream, or from partly or completely dehydrated cream,
52 butter or milk fat) of which not less than 3.5 % of milk fat (European Council, 2000). During
53 processing, the chocolate composition, in terms of type and amount of each ingredient, plays an
54 important role in obtaining an high quality product (Fang and Zhang, 1997; Granger, et al., 2005).
55 In particular, flow properties of cocoa dispersions, a concentrated lipophilic suspension of solid
56 particles dispersed in a continuous fluid, are strongly influenced by their formulations, in terms of

57 solid fraction, fat composition and amount (Attaie et al., 2003; Franke and Heinzelmann, 2008).
58 Sugar particles are very important in chocolate manufacture, of which crystals of a particular size
59 and shape are required. Moreover, if sugar is present in an amorphous state (due to a bad
60 crystallization or to the presence of water) it tends to trap fat, because of its irregular structure
61 (Stortz and Marangoni, 2013), thus increasing the product viscosity. Milk powders (present in milk
62 and white chocolate), with their own physical characteristics and the inner presence of milk fat and
63 lactose, may have also a significant impact on the chocolate processing conditions and on the
64 physical, rheological and organoleptic properties of the final product (Liang and Hartel, 2004).
65 Emulsifiers, if present in low amount of around 0.3-0.5%, can contribute to reduce particle
66 interactions and therefore the viscosity in the chocolate products (Johanson and Bergensthal,
67 1992). As known by literature (Afoakwa et al., 2009; Beckett, 2010; Glicerina et al., 2015a), the
68 structure of chocolate arises both from the various components used in the formulation and from the
69 manufacturing process during the mixing, pre-refining, refining, conching and tempering steps, that
70 strongly determine the different interactions that take place among ingredients. The relationships
71 between all the ingredients present in cocoa dispersions and the continuous phase, influencing the
72 microstructural properties of the final matrix, affect strongly its rheological and textural
73 characteristics in terms of yield stress, apparent viscosity, thixotropy, hardness and consistency
74 (Vavreck, 2004; Schantz and Rohm, 2005). For this reason, microstructure can be considered a
75 fundamental quality parameter for chocolate products. Some authors, in fact, (Braipson-Danthine
76 and Deroanna, 2004; Aguilera, 2005; Varela et al., 2007; Afoakwa et al., 2008) noted that
77 improvements on the quality of existing foods, among which chocolate, and new product
78 formulations require interventions at a microscopic level.

79 In the chocolate manufacture, tempering is an essential step, influencing important product final
80 properties such as colour and hardness and its shelf-life; for this reason the control of process
81 conditions related to this step is of crucial importance for the product final quality and stability
82 (Herrera and Hartel, 2000; Toro-Vazquez et al., 2004; Altimiras, Pyle and Bouchon, 2007; Pérez-

83 Martínez et al., 2007 and Debaste et al., 2008). Several studies (Gosh et al., 2002; Lee et al., 2002;
84 Afoakwa et al., 2008; Beckett, 2010; Svanberg et al., 2011) were performed in order to evaluate the
85 relationships between tempering step and dark chocolate microstructure, appearance, hardness and
86 stickiness. Results showed that a more dense chocolate structure, obtained through an optimal
87 tempering processing, improves the product quality, giving rise to a less fat bloom phenomena
88 during storage. Moreover, an optimal tempering, evaluated according with Nelson (1999) and
89 Afoakwa et al., (2009) by using a computerized tempermeter, gave rise to a chocolate with less
90 hardness and higher values of lightness and gloss, that are considered positive attributes, very
91 appreciated from consumers (Kulozik et al., 2003).

92 To our knowledge, no comparative studies between the main types of chocolate (dark, milk and
93 white) are present in literature. The aim of this research was to evaluate the influence of different
94 formulations on the microstructural, rheological and textural properties of dark, milk and white
95 chocolate types obtained in the same industrial plant. Different rheological models were applied in
96 order to study the rheological behaviour of the chocolate samples.

97

98 **2. Materials and methods**

99 2.1 Materials

100 Dark (D), milk (M) and white (W) chocolate samples were produced in an Italian confectionery
101 factory by using an industrial plant (Buhler, Malmo, Sweden) equipped to produce 6000 kg of
102 chocolate at every production cycle. All samples were obtained after tempering step, at the end of
103 three different processing cycles. Their formulations, that are the standard recipes normally used by
104 chocolate factories are reported in Table 1. After processing chocolate samples were stored in
105 plastic bucket (1 kg capacity) at room temperature until the analytical determinations. Before
106 performing each analysis the samples were melted in a microwave at 150 W for 10 min (Stortz and
107 Marangoni, 2013; Glicerina, et al., 2015a).

108

109 2.2 Methods

110 2.2.1 Microstructural analysis

111 Ten micrographs from each chocolate sample were obtained by using a light microscope (Olympus
112 Optical, Tokyo, Japan) at 10× of magnification. One drop of each sample dispersion (previously
113 diluted with hexane) was placed on a glass slide and carefully covered with a glass slip, placed
114 parallel to the plane of the slide and centred to ensure a uniform sample thickness. Micrographs
115 were captured using a digital camera (Model 2.1 Rev 1; Polaroid Corporation, NY, USA) (Glicerina
116 et al., 2015b). The acquired images were subsequently elaborated using the software Image Pro-plus
117 6.0 (Media Cybernetics Inc., Bethesda, USA). Particles size were measured according with
118 Glicerina et al., (2013), by evaluating the Feret diameter, defined as the distance between two
119 tangent lines to the two opposite sides of the particles (Allen, 1997). The distance between particles
120 was evaluated generating a Euclidian distance map (EDM). The map indicates, for each pixel in the
121 image (black points) of the originally binary picture, the shortest distance between them
122 (Danielsson, 1990; Bayod, 2008). The distance between black points (particles) was expressed as
123 grey values. On the other hand, the white points represented the empty space. For this reason,
124 applying an EDM to the original image it is possible to obtain information about the minimum
125 distance between particles and about the amount and distribution of void spaces (Krislock and
126 Wolkowicz, 2012).

127

128 2.2.2 Fundamental rheological analysis

129 Rheological measurements were carried out at 40 °C using a controlled strain–stress rheometer
130 (MCR 300, Physica/Anton Paar, Ostfildern, Germany) equipped with coaxial cylinders. In steady
131 state conditions, after a pre-shearing of 500 s at 2 s⁻¹, apparent viscosity was measured as a function
132 of increasing shear rate from 2 to 50 s⁻¹ (ramp up) within 180 s, then of decreasing from 50 to 2 s
133 (ramp down); within each ramp 18 measurements were taken (ICA, 2000).

134 In order to study in depth the results of rheological measurements, the obtained flow curves were
 135 fitted by using some specific models, normally employed for concentrated suspensions.

136 The *Casson* model is generally the most used to study the rheological behaviour of chocolate
 137 dispersions, however sometimes it does not reflect in accurate way the physical and rheological
 138 properties of chocolate, especially for low viscosity values. For this reason, further models were
 139 developed such as the *Windhab* model that is recommended for shear rate in the range between 2
 140 and 50 s^{-1} (Ludger and Teixeira, 2007). Another model used for concentrated suspension, especially
 141 in the case of high viscosity, is the *Ostwald de Waele* (Bouzas and Brown, 1995).

142 In preliminary trials the three different models were applied on flow curves obtained from each
 143 sample. The applied model for each chocolate type was chosen on the basis of the best goodness of
 144 fitting obtained. The model of *Ostwald de Waele*, commonly referred to as the *Power Law* model
 145 (Holdsworth, 1993; Hugelsholfer, 2000) is described by the following equation:

$$147 \quad \tau = K^* \dot{\gamma}^n \quad (1)$$

148
 149 where τ is the shear stress (Pa), K is consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (1/s) and n is the
 150 dimensionless flow behaviour index.

151 According to Chevalley (1991), curve points of the *Casson* rheological model represent a better
 152 fitting to chocolate data if the exponent is taken as 0.6 rather than 0.5. This model is described by
 153 the following equation:

$$154 \quad \tau^{0.6} = \tau_0^{0.6} + (n_{PL} \dot{\gamma})^{0.6} \quad (2)$$

155 where τ_0 is the yield stress at the zero point and η_{PL} is the so-called plastic viscosity.

156 The *Windhab* model, recommended (Ludger and Teixeira, 2007) for shear rates in the range
 157 between 2 and 50 s^{-1} at $40 \text{ }^\circ\text{C}$, was used to describe the flow behaviour of W chocolate sample.

158 This model is described by the following equation:

159

$$160 \quad \tau = \tau_0 + \eta_{\infty}^* \dot{\gamma} + (\tau_1 - \tau_0) (1 - e^{-\dot{\gamma} / \dot{\gamma}^*}) \quad (3)$$

161

162 where τ is the shear stress, τ_0 is the shear stress at the zero point, η_{∞} is the infinite viscosity, $\dot{\gamma}$ is
 163 the shear rate, τ_1 is the hypothetical yield stress and $\dot{\gamma}^*$ represents the shear rate corresponding to
 164 the infinite viscosity.

165 By using both the *Casson* and *Windhab* models it is possible to obtain at the same way and
 166 immediately the theoretical viscosity values (η). The *Casson* and *Infinity* viscosities are comparable
 167 because the same basic parameters are involved in these models. As known by literature (Rao,
 168 2007) the *Casson* plastic viscosity can be used as the infinite shear viscosity η_{∞} of dispersions by
 169 considering the limiting viscosity at infinite shear rate.

170 From the *Power Law* model instead the viscosity values are provided indirectly, for this reason
 171 further processing is necessary in order to obtain the theoretical viscosity value. From *Power Law*
 172 model apparent viscosity, according to Ludger and Teixeira (2007), was given, by:

173

$$174 \quad \eta = \text{shear stress/shear rate} = \eta = \frac{\tau}{\dot{\gamma}} \quad (4)$$

175

176 So by replacing $K^* \dot{\gamma}^n$ (equation 1) into the equation (4) it is possible to obtain the following
 177 equation:

178

$$179 \quad \eta = \frac{\tau}{\dot{\gamma}} = \frac{K^* \dot{\gamma}^n}{\dot{\gamma}} = K^* \dot{\gamma}^{n-1} \quad (5)$$

180

181 From the rheological curves of the chocolate samples, the thixotropy values were also obtained.
 182 During shearing, the continuous decrease in apparent viscosity and its subsequently recovery, when

183 flow is discontinuous, create an hysteresis loop (Chhabra, 2006). In this research, thixotropy was
184 evaluated according to Servais et al., (2004), from the difference between viscosity measured at 40
185 s^{-1} during ramp up (from 2 to 50 s^{-1}) and ramp down (from 50 to 2 s^{-1}), multiplied to 40². The
186 thixotropy data obtained in this way, in accordance with the method proposed by Servais et al.,
187 (2004) and Cheng (2003), very accurately represent the values of the hysteresis area underlying the
188 two curves of flow during the increase and decrease of the shear rate.

189

190 2.2.3 Empirical rheological analysis

191 A TA.HDi 500 Texture Analyzer (Stable Micro System Vienna Court, England) was employed to
192 investigate the textural properties of the three different chocolate types. All measurements were
193 performed using a back extrusion test. Samples were analyzed at room temperature, after melting as
194 explained in the section 2.1. The test was carried out in a back extrusion container (50 mm in
195 diameter), filled to 75% with the sample, using a disk (35 mm) attached to a extension bar, with a
196 load cell of 25 kg. The test parameters used were: a pre-test speed of 1 mm s^{-1} , a test speed of 1 mm
197 s^{-1} , a post-test speed of 1 mm s^{-1} and a distance of 30 mm. The following textural properties of
198 sample structure were obtained: consistency (N s), the positive area up to the maximum force
199 during probe descent and cohesiveness (N), the peak maximum of the negative region during probe
200 return (Glicerina et al., 2013)

201

202 2.3 Statistical analysis

203 The rheological curve fitting was obtained by using the software Rheoplus (v.3.0, Anton Paar,
204 Ostfildern, Germany) based on the ordinary least squares statistical method. The results are reported
205 as the average of at least three determinations for each sample. Analyses of variance (ANOVA) and
206 the test of mean comparison, according to Fisher's least significant difference (LSD) were applied
207 on all obtained data. Level of significance was $P \leq 0.05$.

208 The statistical software used was STATISTICA, version 8.0 (StatSoft, Tulsa, Oklahoma).

209

210 **3. Result and discussions**

211 3.1 Microstructural properties

212 In order to study the microstructural properties of chocolate matrices a light microscope was used,
213 since this kind of instrument makes an easy differentiation between particles and void spaces, in
214 relation to the different light diffusion (Kalab et al., 1996). In a conventional bright-field
215 microscope, in fact, illumination is transmitted sequentially through a condenser (the specimen and
216 the objective). Previous researchers (Do et al., 2007; Afoakwa et al., 2008; Afoakwa et al., 2009;
217 Glicerina et al., 2015b) demonstrated the usefulness of this technique to study the particle size, the
218 presence of network and the state of aggregation in dark and white chocolate matrices. In Figure 1
219 micrographs of D, M and W chocolate samples are shown. On the obtained micrographs the
220 characteristics of particle networks and their distribution in the matrices were evaluated. In order to
221 better highlight the state of aggregation of the different matrices and the presence of particles and
222 empty spaces filled with fat, Euclidean distance maps (EDM) were obtained (Figure 2). By using an
223 EDM it was possible to underline the distribution of particles (black areas) and void spaces (white
224 areas), surrounded with fat, in the different matrices and to evaluate the minimum distance between
225 particles and therefore their state of aggregation related to interactions. In Table 2 the particle Feret
226 diameters and the minimum distance between particles of D, M and W chocolate samples are
227 reported. From samples micrographs (Figure 1) and from data reported in Table 2 it is possible to
228 notice how dark and milk chocolate samples (D and M) have greater particles size compared to
229 white chocolate one (W). As known by literature (Sokmen and Gunes, 2006; Beckett, 2009)
230 specific surface area is inversely correlated with particle size and these parameters in chocolate
231 dispersions are strictly related to the amount of fat content in the sample, necessary to obtain
232 desirable flow properties. So usually, smaller are the particles, bigger will be the specific surface
233 area; this implies the presence of more contact points and more interaction between particles
234 (Chevalley, 1991). For these reasons only considering particle size results we would expect the

235 highest rheological values (in terms of yield stress, viscosity and thixotropy) in white chocolate
236 sample; however microstructural and hence rheological properties of chocolate samples are affected
237 not only by the particle size, but also by other factors, including the amount and distribution of fat,
238 presence of emulsifiers and solid particles, and particles shape. Therefore, in the case of the three
239 different formulations, the quality and quantity of ingredients become very important for the
240 products final characteristics.

241 In sample W, made up from smallest particles size, a less dense crystalline network and reduced
242 particle-particle interactions are present, parallel to the presence of highest distance between
243 particles filled with fat. According with the studies of Afoakwa et al., (2008), high fat content in a
244 suspension tends to wet the matrix, opening up the fat phase, that fill the voids within the crystal
245 network reducing resistance to flow. Sample M, even if had particles with diameters (Table 2) not
246 statistically different from sample D, showed a less aggregate structure, with more open spaces
247 (filled with fats) than sample D (Figure2), that had the lowest minimum distance value between
248 particles. Milk chocolate sample showed values of this parameter intermediate and significantly
249 different from D and W (Table 2). The fat in the voids derives both from the cocoa butter, present in
250 all chocolate types, and from milk fat presents in milk powders in M and W samples. Moreover, in
251 both M and W chocolate samples, lactose (the major carbohydrate of whole milk powders) if
252 present in crystalline forms (Aguilar et al., 1994; Aguilar and Ziegler, 1995; Koc et al., 2003;
253 Lonchamp and Hartel, 2004), contributes to make available a part of milk fat, normally entrapped
254 in milk powders, so increasing its amount in the final product. The fat quantity of sample M and W
255 was quite similar (Table 1), however, the more aggregated microstructure of M sample compared to
256 W (Figure 1, Table 2) is probably due to the presence of cocoa particles between sugar ones and
257 probably the absence of lecithin in M sample formulation, that usually migrates to sugar/fat
258 interfaces and coats sugar crystals, promoting the dispersion of the latter (Johanson and
259 Bergensthal, 1992; Vernier, 1998).

260 As shown by micrographs (Figure 1) it is possible to notice how D and M samples present more
261 evidenced particles with an irregular and flaky shaped than W; sugar particles are mainly evidenced
262 in M sample micrograph.

263

264 3.2 Fundamental rheological properties

265 In Figure 3 the flow curves of dark, milk and white chocolate samples are reported. As shown,
266 viscosity values decreased with the increase of the shear rate in all samples, underlining the
267 presence of a shear thinning behaviour (Izidoro et al., 2008). All chocolate samples exhibited a
268 non-ideal plastic behaviour with a yield stress (related to amount of energy required to initiate fluid
269 flow) and plastic viscosity (energy required to keep fluid in motion) typical of a non-newtonian
270 liquid (Ziegler and Hogg, 1999; Afoakwa et al., 2008; Beckett, 2010).

271 In particular, sample D showed the highest apparent viscosity (Figure 3) with an initial value of
272 around 7 Pa s, followed by sample M with initial apparent viscosity values of around 5 Pa s; sample
273 W had the lowest apparent viscosity value. In order to better highlight differences between the three
274 chocolate samples, yield stress and plastic viscosity were obtained from flow curves, respectively at
275 5 s^{-1} and 30 s^{-1} of shear rate, according with ICA (2000) and Servais et al., (2004). Obtained values
276 are reported in Table 3. Dark chocolate (D) showed the significantly highest yield stress and
277 viscosity values ($p < 0.05$) compared to M and W samples. This means that the amount of energy
278 needed to start flow was the highest in the former. These results are in agreement with those
279 obtained from the microstructural analysis, sample D in fact showed a more aggregate matrix than
280 the others (Figure 2), having the lowest amount of cocoa butter in formulation (Table 1). Sample M
281 had intermediate values of yield stress and viscosity, between those of D and W. M chocolate had in
282 formulation more fat than D; the lubricating effect of fat (Beckett, 2010) is the cause of its lower
283 rheological parameters. The lowest viscosity and yield stress values of sample W can be attributed
284 both to the highest quantity of fat (from cocoa butter and milk fat) and to the presence of lecithin in
285 its formulation, that, as known by literature (Vernier, 1998; Afoakwa et al., 2008; Beckett, 2010),

286 contributes to reduce particle-particles interaction. Moreover, as mentioned before in the
287 microstructural section, the presence of crystalline lactose in M and W samples must be considered
288 as a factor that could have influenced their lower viscosity values, promoting the release of
289 entrapped milk-fat. Rheological behaviour of chocolate flow curves was further studied by applying
290 different rheological models. In particular, D sample flow curves were well fitted by applying the
291 *Ostwald de Waele* model (Rao, 2007); M chocolate curves by using the *Casson* model (ICA, 1973),
292 modified by Chevalley (1991) and W chocolate curves by using the *Windhab* model (IOCCC,
293 2000). The fitted constants of each rheological model are reported in Table 4.

294 Very high coefficients of determinations ($R^2 = 0.99$) were obtained in all cases, demonstrating that
295 the chosen models for each chocolate sample fit well the related data. In Table 5, the obtained
296 apparent viscosity values for each sample are reported. Also in this case, and in agreement with the
297 previous reported results, D chocolate sample had the highest apparent viscosity value (9.62 Pa s).
298 Chevalley (1991) and Afoakwa, Paterson, Fowler & Vieira (2007) reported that higher viscosity
299 values are related with more aggregate matrices with less voids space between particles, probably
300 due to a low amount of fat that promotes particle-particle interactions.

301 The highest value of thixotropy showed by sample D (Figure 4) demonstrates further the more
302 aggregate structure of this kind of chocolate compared to the M and W ones. High thixotropic
303 values in fact are due to the high damage of the structure, highlighted immediately after the stress
304 removal, which can be attributed (Aguilera and Stanley, 1999; Afoakwa et al., 2008) to a high level
305 of matrix aggregation, which undergoes to an irreversible break. Sample M showed an intermediate
306 thixotropic value between D and W ones. As expected, W chocolate, having the highest amount of
307 fat and hence a more fluid matrix and a less aggregate structure, showed the less intense thixotropic
308 behaviour, being able to recovery most part of its initial structure.

309

310 3.3 Empirical properties

311 The textural parameters of consistency and cohesiveness were chosen being the most related to the
312 sensory mechanical properties of chocolate during consumption (Beckett, 2010). The consistency
313 and cohesiveness values of chocolate samples, obtained by using a *back extrusion* test, are reported
314 in Table 6. D chocolate sample presented the highest and significantly different values of both
315 textural parameters; these results further confirm that this sample was characterized by a very
316 aggregate and dense structure, that makes more resistance to the probe return during the back
317 extrusion test. M and W chocolate samples showed the lowest consistency and cohesiveness values,
318 underling the presence of a structure with weaker interactions between particles.

319

320 **4. Conclusions**

321 The obtained results showed how different ingredients in chocolate recipes affect in strong way
322 microstructural and rheological properties of the final product. In particular, different amount of fat
323 involves changes in the particle-particle interaction, in terms of distance between particles and their
324 distribution, as well as, the solid fat and non-fat particles. The presence of lecithin and crystalline
325 lactose, can also influence the final product properties.

326 Lower cocoa butter concentrations, parallel to high fraction of solid particles, such as in the dark
327 chocolate formulation, promote particle-particle interactions, involving higher values of rheological
328 characteristics. On the other hand, higher cocoa butter amount, even if in presence of higher amount
329 of non fat particles (sugar), together with the presence of milk fat (from milk powders), reduce
330 resistance to flow. This effect was shown in white chocolate sample, made up of smallest particles
331 having the highest distance between them. Moreover, the synergistic effect of the lecithin, that
332 promotes the reduction of particles inter-forces, involves a further reduction in the yield stress,
333 viscosity and thixotropic values.

334 The influence of each single ingredient, in terms of type and amount, must be taken into account in
335 order to improve or modify the micro- and macrostructure characteristics and hence the final quality
336 of chocolate products.

337

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340

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1 **Table 1.** Dark, milk and white chocolate formulations.

Ingredient	Dark chocolate	Milk chocolate	White chocolate
	(%)	(%)	(%)
Sugar	39.52	47.00	47.00
Cocoa liquor	53.00	7.00	–
Cocoa butter (further added)	7.00	25.00	31.00
Full cream milk powder	–	21.00	21.50
Lecithin	0.30	–	0.49
Sodium carbonate	0.15	–	–
Vanilla extract	0.03	–	0.01

2

- 1 **Table 2.** Particles dimension (Ferret diameter) and minimum distance between particles of dark (D),
2 milk (M) and white (W) chocolate samples.

Samples	Feret diameter (μm)	Minimum distance between particles (μm)
D	$20.16 \pm 2.17^{\text{a}}$	$12.64 \pm 2.67^{\text{c}}$
M	$19.30 \pm 2.32^{\text{a}}$	$16.25 \pm 1.92^{\text{b}}$
W	$14.27 \pm 2.29^{\text{b}}$	$23.46 \pm 2.34^{\text{a}}$

- 3 Values (mean \pm standard deviation) in the same column followed by different letters differ significantly at $p < 0.05$ level

1 **Table 3.** Yield stress and viscosity values, calculated respectively at 5s^{-1} and 30s^{-1} of shear rates, of
2 dark (D), milk (M) and white (W) chocolate samples.

Samples	Yield stress	Apparent Viscosity
	(Pa s)	(Pa s)
D	33.07 ± 0.15^a	6.87 ± 0.03^a
M	14.56 ± 1.78^b	1.32 ± 0.12^b
W	11.50 ± 0.14^c	0.98 ± 0.02^c

3 Values (mean \pm standard deviation) in the same column followed by different letters differ significantly at $p<0.05$ level

1 **Table 4.** Rheological constants of dark, milk and white chocolate samples obtained respectively
 2 from Power law, Casson and Windhab models.

Rheological models	Dark chocolate (D)	Milk chocolate (M)	White chocolate (W)
Power Law			
<i>Consistency Index (k), Pa sⁿ</i>	4.78±0.22		
<i>Flow behaviour Index (n)</i>	0.45±0.04		
<i>*Determination coefficient (R²)</i>	0.99		
Casson			
<i>Yield stress (τ_0), Pa</i>		3.68±0.15	
<i>Plastic viscosity (η_{PL}), Pa s</i>		0.68±0.04	
<i>*Determination coefficient (R²)</i>		0.99	
Windhab			
<i>Yield stress at zero point (τ_0), Pa</i>			0.00±0.00
<i>Infinite viscosity (η_∞), Pa s</i>			0.59±0.07
<i>Hypotetical yield stress (τ_1), Pa</i>			8.32±0.18
<i>Shear rate of infinity viscosity ($\dot{\gamma}$), s⁻¹</i>			1.04±0.01
<i>*Determination coefficient (R²)</i>			0.99

4 *p ≤ 0.01

5 Values are means of three replicate experiments ± standard error (SE)

6

1 **Table 5.** Apparent viscosity of dark (D), milk (M) and white (W) chocolate samples, evaluated by
2 applying respectively *Power Law*, *Casson* and *Windhab* models.

Samples	Viscosity (Pa s)
D	9.62±0.38 ^a
M	1.55±0.33 ^b
W	0.59 ±0.23 ^c

3 Values (mean±standard deviation) in the same column followed by different letters differ significantly at p<0.05 level
4

1 **Table 6.** Consistency and cohesiveness index of dark (D), milk (M) and white (W) chocolate
2 samples.

Samples	Consistency	Cohesiveness
	(N s)	(N)
D	110.14±13.96 ^a	16.07±1.25 ^a
M	1.22±0.16 ^b	3.16±0.53 ^b
W	0.87±0.06 ^b	1.83±0.37 ^b

3 Values (mean±standard deviation) in the same column followed by different letters differ significantly at p<0.05 level
4

1 **Figure Captions**

2

3 Fig. 1 Micrographs of chocolate samples: dark (D), milk (M) and white (W).

4

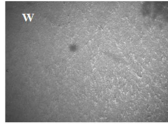
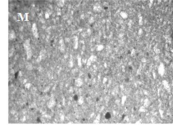
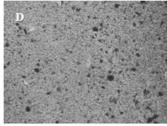
5 Fig. 2 Euclidean Map of chocolate samples: dark (D), milk (M) and white (W).

6

7 Fig. 3 Flow curves of chocolate samples: dark (D), milk (M) and white (W).

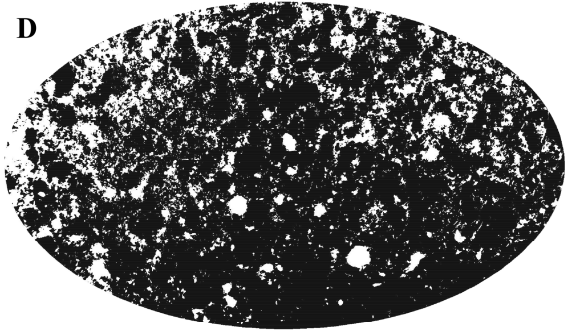
8

9 Fig. 4 Thixotropy of chocolate samples: dark (D), milk (M) and white (W).

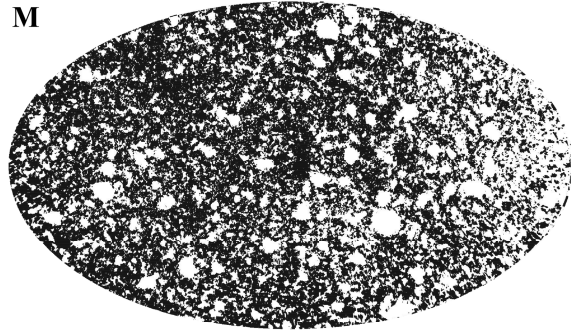


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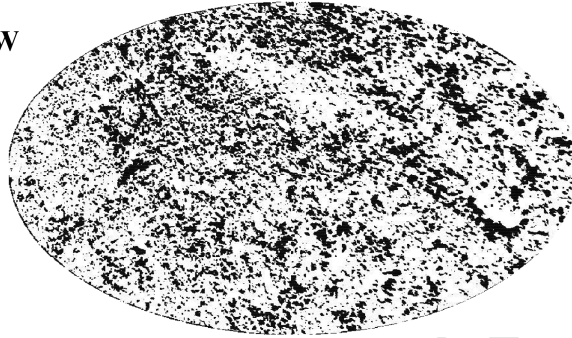
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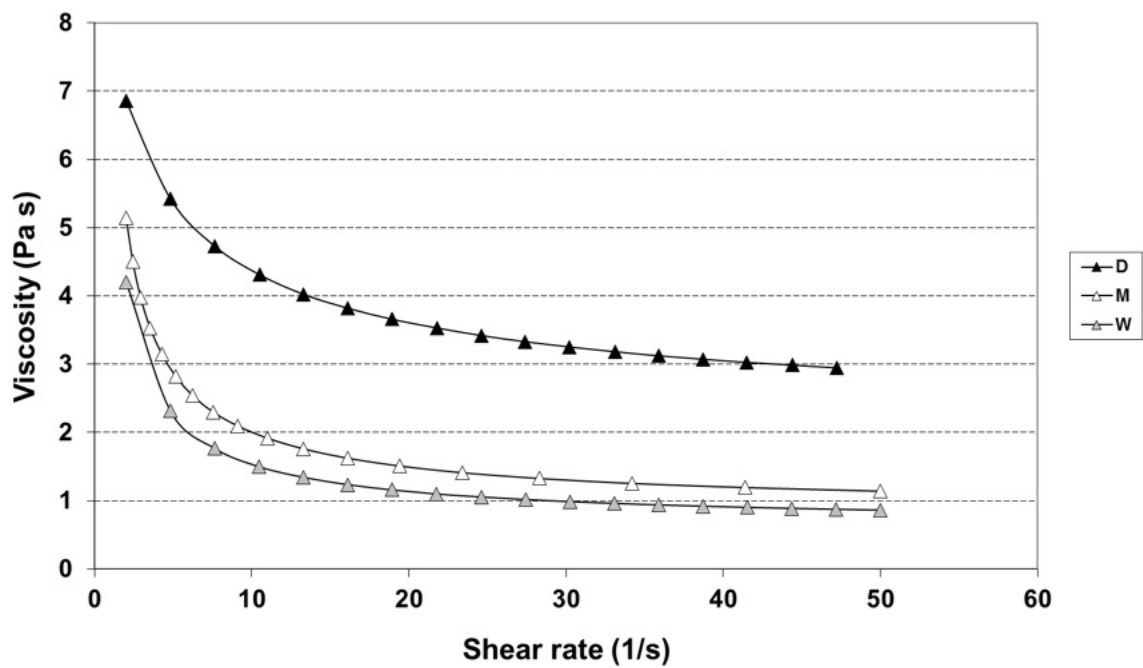
M

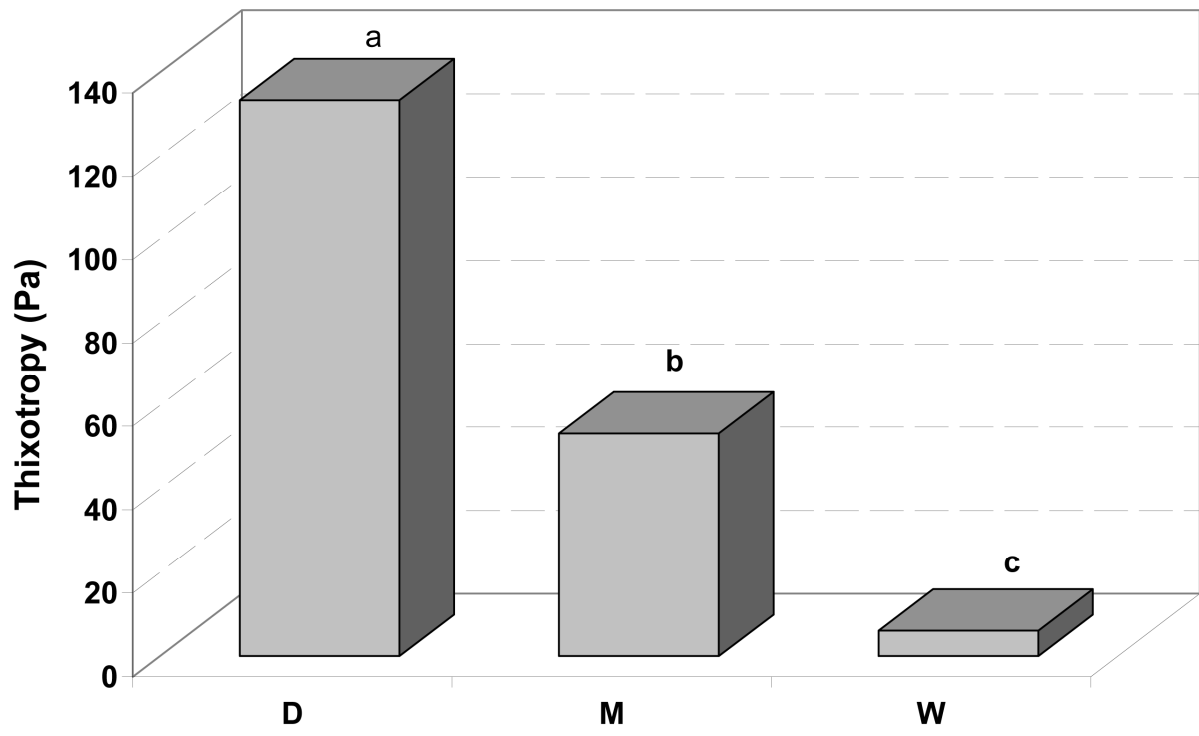


W



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Highlights

- Formulation influences dark, milk and white chocolate characteristics
- Power law, Casson and Windhab models successfully fit rheological data
- Microstructural and rheological analysis well discriminated chocolate types