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THE INFLUENCE OF PROCESS STEPS ON MICROSTRUCTURAL, RHEOLOGICAL AND THERMAL PROPERTIES OF DARK CHOCOLATE
UTICAJ PROCESNIH KORAKA NA MIKROSTRUKTURNE, REOLOŠKE I TERMIČKE OSOBINE TAMNE ČOKOLADE

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ABSTRACT

The aim of this research was to investigate the influence of different process steps on microstructural, rheological and calorimetric properties of dark chocolate. Samples were obtained at each phase of the manufacturing process: mixing, pre-refining, refining, conching and tempering. Microstructural properties, fundamental rheological parameters (yield stress, apparent viscosity, G' and G") and thermal ones (Tonset, Tend and ΔH) were evaluated by using an environmental scanning electron microscope (ESEM), a controlled strain – stress rheometer and a differential scanning calorimetry (DSC) respectively. ESEM analysis revealed an increase of the aggregation and contact point between particles from mixing to refining step, that underwent a drastic raising of all considered rheological (yield stress, apparent viscosity, G', G'') and thermal parameters (Tonset, Tend, ΔH). Samples obtained from the conching and tempering steps were characterized by less dense aggregate structure and lower particle–particle interactions, due to the adding of further cocoa butter and lecithin. The addition of fat and lecithin in fact covering the sugar and cocoa particles, reduced interactions and caused a decrease in all rheological and thermal parameters.

***Keywords**: dark chocolate, process steps, microstructure rheological properties, thermal properties.*

REZIME

Cilj ovog istraživanja bio je da se ispita uticaj različitih procesnih koraka na mikrostrukturne, reološke i kalorimetrijske osobine crne čokolade. Uzorci su uzeti u svakoj fazi proizvodnog procesa: mešanje, pre- prerada, prerada, končiranje i kaljenje. Mikrostrukturne osobine, osnovni reološki parametri (napon, viskoznost, G 'i G ") i termičke osobine (Tonset, Tend i ΔH) ocenjeni su korišćenjem elektronskog mikroskopa (ESEM), kontrolisani reometarom i diferencijalnim kalorimetrom (DSC). ESEM analiza pokazala je povećanje agregacije i kontakt tačka između čestica od mešanja do pripreme, koje su pretrpele drastičan porast svih razmatranih reoloških (napon, viskoznost, G ', G") i termičkih parametara (Tonset, teže, ΔH). Uzorke iz procesa končiranja i kaljenja karakterisale su niža gustina i niža čestica-čestica interakcija, zbog dodavanja kakao butera i lecitina. Dodavanjem masti i lecitina u stvari pokriva šećer i kakao čestice, smanjuje interakcije i izazva pad svih reoloških i termičkih parametara.

***Ključne reči**: crna čokolada, procesni koraci, mikrostrukturna reološka svojstva, termička svojstva.*

INTRODUCTION

Dark chocolate can be defined as a concentrated suspension made up of solid particles (sugar and ground cocoa particles) dispersed in a Newtonian fluid, generally cocoa butter (*Afoakwa et al., 2008a*). The physico-chemical and microstructural properties of chocolate depend on many factors besides the ingredients and their proportions. The different process steps (mixing, pre-refining, refining, conching and tempering) of chocolate manufacture and the different adopted process parameters can affect chocolate properties which, in turn, determine the behaviour and the characteristics of the final product. The processing of foods, dark chocolate in particular, brings several changes in their microstructure. During chocolate manufacturing, mixtures of sugar, cocoa and fat are heated, cooled, pressurized and refined (*Beckett, 2008*). These steps break agglomerates and distribute lipid and lecithin-coated particles through the continuous phase, modifying the microstructure of final chocolate (*Afoakwa et al., 2009a*). Since the macroscopic properties of food, as rheological and thermal attributes are strongly determined by the microstructure, an evaluation of the product microstructure is a necessary prerequisite for understanding its macroscopic properties (*Bayod, 2008*). In particular rheology properties are important because related both to the efficiency of the main steps in the process (mixing, pumping, transportation, etc.) (*Servais et al., 2002*), and to the final quality of product (*Ahmed & Ramaswamy, 2006*). For this reason the knowledge of the relations between microstructure, rheological and thermal properties could be very useful in order to optimize the final properties of chocolate. Several authors (*Servais et al., 2004; Afoakwa et al., 2008a; 2008b; Baldino et al., 2010; De Graef et al., 2011; Efraim et al., 2011; Fernandez et al., in press*) have investigated the microstructural and physico-chemical properties of dark chocolate and in particular the rheological and thermal ones. Despite of the interesting obtained results, in all the previous cited works only the characteristics of finished product was taken into account. In our opinion each manufacture step (mixing, pre-refining, refining and conching) cause drastic modification in the product microstructure influencing its final properties. Understanding how the single process step can affect the microstructural properties of dark chocolate, could be very useful in order to manipulate and optimize the process efficiency and mainly to predict and improve quality of final product. For this purpose in the present work the microstructural, rheological and thermal properties of dark chocolate were evaluated throughout the manufacturing process.

MATERIAL AND METHOD

Dark chocolate samples were produced in an Italian confectionery factory using an industrial plant (Buhler, Malmo, Sweden) provided of mixer, pre-refiner, refiner, conching and tempering machine, equipped to produce 6000 Kg of chocolate at every production cycle. Dark chocolate production was made up by different steps: mixing, pre-refining, refining, conching, tempering, demoulding and packing. The formulation employed for the recipe was: cocoa liquor (53%), sugar (39.52%), cocoa butter (7% added during the conching step), soy lecithin (0.3%), sodium carbonate (0.15%) and vanilla extract (0.03%). Experimen-

tal samples were taken after each production phase: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E). Samples were stored in plastic bucket (1 Kg capacity) at room temperature until the analytical determinations. Before performing each analysis the samples were melted in a microwave at 150 W for 25 minutes. The melting parameters were chosen after preliminary experiments in order to avoid changes in the chocolate properties.

Microstructure analysis

Samples microstructure was observed using an environmental scanning electron microscope ESEM (Evo 50 EP, Zeiss, Germany) equipped with a microprobe (EDS Mod. 350, Oxford Instrument, UK). The detector used was a backscatter electron detector (QBSE) that provided good compositional contrast imaging at 20 kV and in low vacuum mode with 100 Pascal at 500x magnification, taking 10 micrographs for each samples. These parameters were chosen after preliminary trials and according to *Dahlenborg et al.* (*2010*)*,* considering that they cause minimal damage of the chocolate surface and in order to optimize the imaging quality. The acquired images were subsequently elaborated using the software Image Pro-plus 6.0 (Media Cybernetics Inc Bethesda, USA).

Fundamental rheological properties

Measurements were carried out at 40 °C using a controlled stress-strain rheometer (MCR 300, Physica/ Anton Paar, Ostfildern, Germany) equipped with a system of coaxial cylinders (CC27). The rheological behaviour of dark chocolate was analyzed in steady state and dynamic conditions. In steady state conditions, after a pre-shearing of 500 s at 5 s^{-1}, viscosity was measured increasing shear rate from 2 to 50 s^{-1} within 180 s, taking 18 points measurements (*ICA, 2000*). The yield stress (Pa) and the apparent viscosity (Pa s) were obtained according with *ICA* (*2000*) and *Servais et al., (2004)* evaluating the values of stress respectively at shear rates of 5 and 40 s^{-1}. In dynamic conditions, oscillatory tests were performed to investigate the viscoelastic properties of samples and to evaluate the storage (G') and the loss (G") modulus. In order to identify the linear viscoelastic range (LVR), in which the viscoelastic properties are independent from the stress conditions, stress sweep tests were applied. Frequency sweep tests were carried out in the viscoelastic linear region at the constant deformation amplitudes of 0.007%, previously evaluated with the stress sweep test, in the range from 100 to 1 Hz.

Thermal properties

Melting properties of dark chocolate were evaluated by using a differential scanning calorimeter (Pyris DSC Series 6, Perkin Elmer Corporation, Wellesley, USA). Adopting the method reported by *Afoakwa et al.,* (*2009b)* DSC was calibrated by using indium (melting T 156.60 °C, ΔH 28.71 J/g) and tin (melting T 231.93 °C, ΔH 60.46 J/g) at a scan rate of 10 °C/min using an aluminium pan as reference. Samples (5 mg) were loaded into 40 ml capacity pans with holes and sealed using a sample press. Pans were heated at 10 °C/min from 15 to 200 °C in a N_2 stream. Onset temperature (T onset), end temperature (T end) and enthalpy of melting (ΔH) were calculated for each peak present in the thermogram obtained (*Gloria and Sievert, 2001*).

Statistical analyses

Analyses of variance (ANOVA) and the test of mean comparison according to Fisher least significant difference (LSD) were applied. Level of significance was $P \leq 0.05$.

RESULTS AND DISCUSSION

In Figure 1 (a, b, c, d, e) the micrographs related to the samples A, B, C, D, E obtained by ESEM are shown.

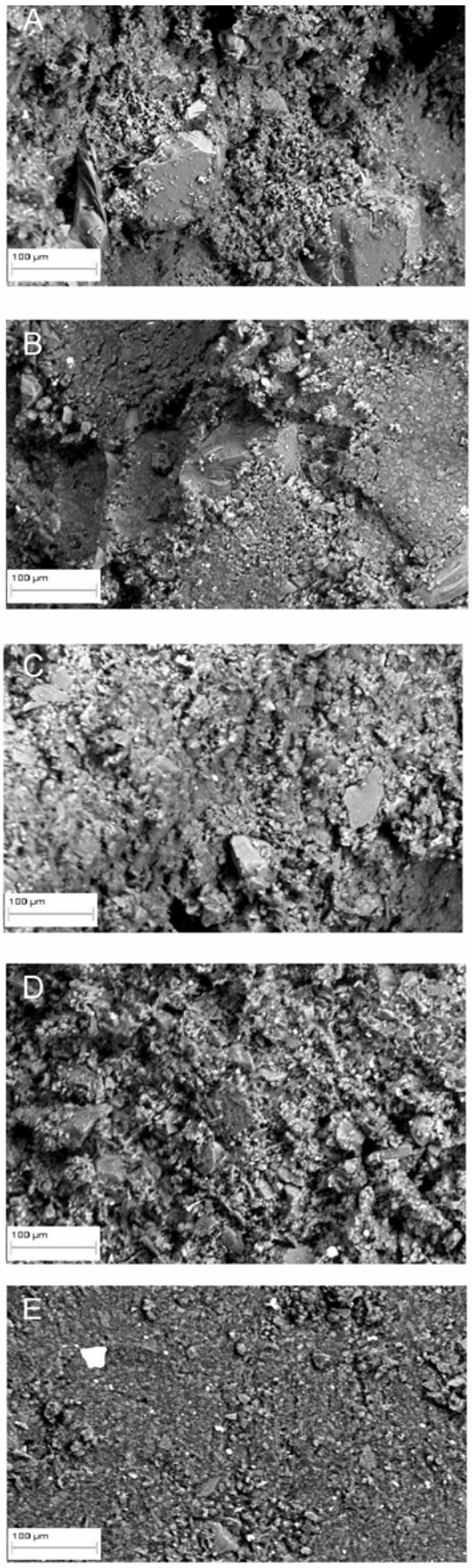


Fig. 1. Microghraphs of dark chocolate after different processing steps: (A) Mixing, (B) Pre-refining, (C) Refining, (D) Conching and (E) Tempering. Scale(a) -(e) 100µm

Electronic microscopy was used to characterize the variations, during the different processing steps, in sugar crystalline network, particle-particle interaction and particle-fat phase behaviour of chocolate samples. Microstructural examination showed a clear decrease of samples particle size from the mixing step (A) to the pre-refining one (B), until the refining phase (C) (Figure 2. a, b, c). The reduction of the particle diameter causes an increase of the particle number, parallel to an increase of contact points between particle-particle, due to chemical and mechanical interactions, according to *Servais et al.,* (*2004*) and *Afoakwa et al.,* (*2009a*). The increase of particle interactions from sample A to C caused a reduction of the particles mobility due to their high aggregation. The result is a fully dense and packed suspension in which small particles fill spaces between large ones *(Bayod, 2008)*. Samples D and E (Figure 2 d, e), having particles with smaller size diameter, were constituted by a less dense sugar crystalline network, highlighted by a larger number of void spaces. This could be related both to the further addition of cocoa butter during the conching step that, (*Beckett, 2000*; *Afoakwa, 2009a),* wets the suspension filling the voids within the crystal network and opens the structure, and also to the addition of lecithin. Lecithin migrates to sugar/ fat interfaces and coats sugar crystals, reducing particle-particle interactions and scattering crystals in the fat phase (; *Dhonsi and Stapley, 2006; Beckett, 2008*). In Figure 2 the flow curves of the dark chocolate samples, obtained increasing the shear rate from 2 to 50 s^{-1} are reported.

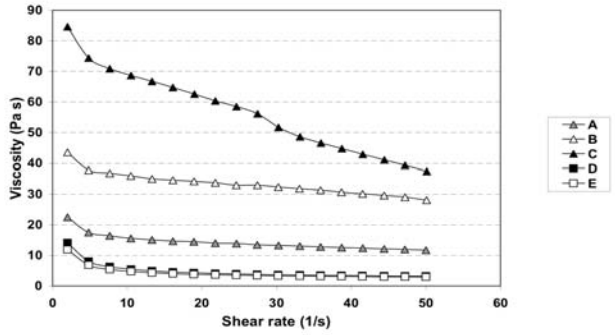


Fig. 2. Changes of viscosity (Pa s) of dark chocolate samples, during mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps

All samples exhibited a typical non Newtonian behaviour, characterized by dependence of viscosity upon of flow conditions (*Chabbra, 2006*). In particular, apparent viscosity of these products decreases with the increase of the shear rate, indicating pseudoplasticity. As illustrated by Figure 2, sample C presents the highest values of viscosity with initial values ranging between 80 and 90 Pa s, followed by samples B with initial viscosity values between 40 and 50 Pa s and sample A with values between 20 and 30. The lowest viscosity values belong to samples D and E obtained from the last two steps of the manufacture process. In order to better explain the rheological values obtained by the flow curves, in Table 1 are shown the values of yield stress and apparent viscosity.

Rheological considered values showed a significantly increase from sample A to sample C. This could be explained considered results obtained from the microstructural examination. The passage from the mixing steps through the pre-refining (B) and refining (C) ones involve a reduction in the particle size that causing an increase in the contact points between them, forms very aggregate structures. According with *Bayod, 2008*; in these conditions samples need of major amount of stress to break them and initiate to flow. Samples D and E present the lowest values of viscosity parameters probably related with their less aggregate packing structure network. In these steps, in fact the addition of lecithin and further cocoa butter, because of their lubricating action, reduced the particle–particle interactions, increasing their mobility that involved a reduction of viscosity (*Vernier, 1998*). These results are supported by the studies of *Coussot and Ancey,* (*1999*) and *Larsoon, (1999)* that noticed an high dependence of yield stress and apparent viscosity on particles size and their interaction.

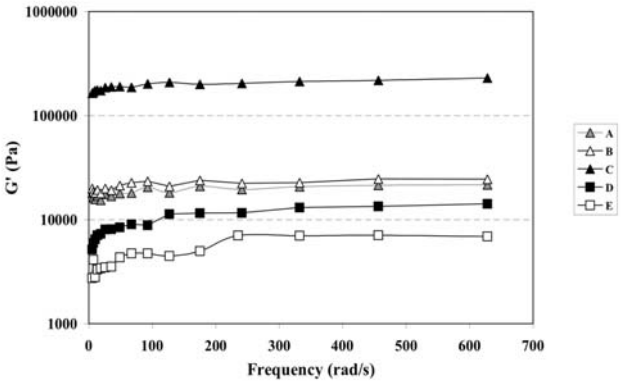
Table 1. Yield stress (Pa) and apparent viscosity (Pa s) of dark chocolate samples

Samples	Yield stress (Pa)	Apparent Viscosity (Pa s)
A	84.50 ^{b}	586.67 ^{b}
B	182.33 ^{c}	1406.70 ^{c}
C	358.67 ^{d}	1880.00 ^{d}
D	38.77 ^{a}	161.67 ^{a}
E	33.07 ^{a}	147.33 ^{a}

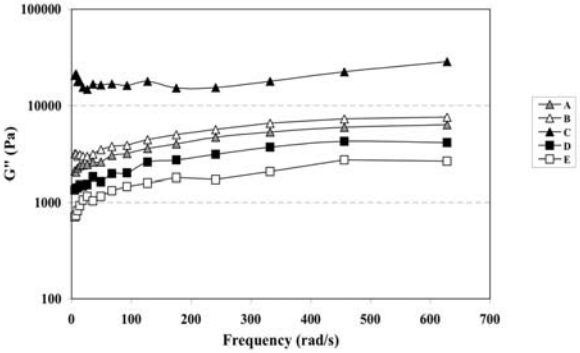
a-d values in the same column followed by different letters differ significantly ($p < 0.05$).

Stress sweep tests were performed in order to identify the linear viscoelastic region (LVR), in which properties of material are independent from the stress conditions. The results (data not shown) showed a narrow viscoelastic region, low intensity stresses can destroy the structure of system.

Results of frequency sweep test in terms of storage and loss modulus, are reported in Figures 3 a, b.



a)



b)

Fig. 3. Storage (a) and loss (b) modulus of dark chocolate samples.

Storage modulus values (G') are higher than loss modulus (G") ones for all samples, indicating that all dark chocolate samples had a solid, elastic-like behaviour. This suggests that under

non destructive conditions the elasticity has a predominant effect on viscosity (*Peressini et al., 2006*). Samples B and C showed the highest G' values, due to their aggregate structure according with ESEM results. Sample A, showed viscoelastic properties with intermediate values of G' and G" inside the clusters B-C and D-E. Some authors (*Johansson and Bergensthal, 1992*) observed that a high value of G' is related to a high level of interactive forces between particles; this confirms the high amount of stress required by samples B and C to start flow (Figure 1). The lowest parameters of G' and G" were found for the samples D and E, constituted by a weakly structured system, due to the lubricating and emulsifier effect of fat and lecithin. These results are in agreement with the studies of *Johansson and Bergensthål* (*1992),* that highlighted how the effect of emulsifiers on the sugar particles, reducing the changing in the interaction particles and in the network structure ones, involves a decrease of the elastic component G'. In Table 2 are reported the values of T onset, T end and ΔH of dark chocolate samples obtained by heating all chocolate samples from 15 to 200 °C.

Table 2. Melting properties by DSC measurements of dark chocolate samples after mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) steps

Samples	FAT			SUGAR		
	T onset (°C)	T end (°C)	ΔH (J/g)	T onset (°C)	T end (°C)	ΔH (J/g)
A	24.323[a]	34.011[a]	38.960[a]	174.89[a]	189.98[a]	30.015[a]
B	30.614[c]	36.657[c]	45.378[b]	176.36[a]	190.79[a]	46.841[b]
C	30.538[c]	37.222[c]	51.464[c]	180.97[b]	192.30[b]	48.954[c]
D	26.011[b]	35.059[b]	37.696[a]	175.72[a]	189.68[a]	29.229[a]
E	25.667[b]	35.344[b]	37.769[a]	174.73[a]	190.13[a]	28.685[a]

a-d values in the same column followed by different letters differ significantly ($p < 0.05$)

It is known that Tonset corresponds to the temperature at which a specific crystal form starts to melt; T end represents the temperature of the complete melting and ΔH the amount of energy required to complete the liquefaction (*Afoakwa et al., 2008b*).

Statistically differences between all samples were highlighted regarding Tonset and Tend of fat (cocoa butter) melting. An increase in the Tonset, Tend and ΔH was noted from sample A to C. Samples B and C, constituted by a very aggregate structure, as shown previously, probably needed higher temperature to start and finish their fat melting than sample A. In this sample the absence of an aggregate structure and the presence of large not refined particles, provides less resistance to breakage and melting and are probably the cause of the lowest obtained values of fat T onset and T end. A significantly decrease of these parameters was instead noted in samples D and E, due to the emulsification effect of cocoa butter and lecithin. As far as the sugar melting is concerned, only sample C showed Tonset and Tend values significantly different from those of the others samples.

The ΔH values of both fat and sugar, were higher in samples B and C compared to the other dark chocolate samples, confirming an higher request of energy in order to complete the sugar and fat melting, due to the existence of very consistent structures (*Afoakwa et al., 2009b*).

CONCLUSION

ESEM analysis was very useful in order to discriminate the differences existing at microstructural level between dark chocolate samples, highlighting the increase of small particles number from the mixing to the refining step, that involves a reduction of the particles mobility due to their high aggregation. Microstructural results were strictly related to the rheological and thermal ones, in fact from the mixing to the refining step there was a drastic increase of all considered rheological (yield stress, apparent viscosity, G', G'') and thermal parameters (Tonset, Tend, ΔH). Obtained results show that the knowledge of the changes occurring in the product matrix at each manufacturing stage could be very useful in order to optimize the manufacture process efficiency and to improve the quality of final product.

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