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Effect of ball milling on physical properties of cocoa powder

Maurizio Asfalto[1], Marco Faieta[1]*, Sarisa Suriyarak[2], Paola Pittia[1]

[1]Department of Biosciences and Technology for Food Agriculture and Environment, University of Teramo, Teramo, Italy; [2]Department of Food Technology, Faculty of Food Science, Chulalongkorn University, Bangkok, Thailand

***Corresponding Author:** Marco Faieta, Department of Biosciences and Technology for Food Agriculture and Environment, University of Teramo, Teramo, Italy. Email: mfaieta@unite.it

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Abstract

Ball milling (BM) was applied to cocoa powder with varying fat content to improve suitability for developing innovative beverages. Cocoa powder is widely used, but the limited water dispersibility and/or stabilizing ability of emulsified systems restricts its applications in food design. BM treatments applied differed in treatment time (10, 20, or 30 min) and rotation speed (450 or 550 rpm). After BM, particle size increased or decreased (39.7 and 22.3% respectively, on average) depending on cocoa composition and processing conditions. This corresponded to changes in microstructure, lipid structure, color, and bioactive content with promising results for novel ingredient development.

Keywords: ball milling; cocoa powder; dispersion degree; fat content; quality

Introduction

Cocoa is obtained from the seeds of the cocoa tree (*Theobroma cacao*). Originally it was consumed by the Aztecs and the Mayans in the form of a spiced drink called "Chocolatl" (Varnam and Sutherland, 1994). Cocoa products and beverages are consumed globally for their rich flavors and health benefits as cocoa is rich in bioactive compounds such as flavonoids and methylxanthines. These compounds have strong antioxidant effects which help reduce oxidative stress and lower the risk of chronic disease (Cinar *et al*., 2021; Crozier *et al*., 2014). Cocoa bean processing is necessary to develop the characteristic color, taste, and flavor while reducing the astringency and bitterness, which are desirable in cocoa-based products. The processing phase includes fermentation, drying, cleaning, shell removal, and roasting which are the primary technological steps. During processing, various chemical reactions occur causing main changes in the chemical composition with the development of the characteristics cocoa sensory properties (Paulik *et al*., 2019).

Cocoa powder is a key ingredient derived from the chocolate industry, produced from cocoa beans that have undergone fermentation, drying, and roasting, followed by the partial extraction of cocoa butter. (Norton *et al*., 2019). For beverage production, cocoa powders with reduced cocoa butter content (8–10%) are generally used (Barisic *et al*., 2023). Moreover, cocoa powder is often subjected to alkalization, an optional process aimed to neutralize cocoa acidity to pH 7. This operation affects physical and chemical characteristics of the cocoa by darkening its color, reducing the bitterness, and increasing its solubility (Germann *et al*., 2019; Valverde *et al*., 2020) making the final product more suitable for the formulation of beverages.

The cocoa powder is composed of carbohydrates and fibers (26–40%), proteins (15–20%), carbohydrates (≈15%), lipids (10–24%), minerals, vitamins, and a variety of bioactive compounds, such as phenolics, hydrocolloids, and theobromine (Adeyeye, 2016). The lipidic fraction of cocoa powder contributes to the sensory and

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physical properties such as shear flow and dispersibility of the product (Petit *et al*., 2017; Shittu and Lawal, 2007). The crystallization behavior of cocoa fat in the solid state affects the sensory and rheological properties of cocoa products.

In the food industry, cocoa powder is primarily used for flavoring in confectionery, bakery products, and frozen desserts, while also enhancing color, stability, and texture in colloidal systems such as ice cream, desserts, and chocolate beverages. In the latter case, ready-to-drink cocoa products and premixes for easy preparation are widely available. The quality of cocoa powder suspensions depends on particle size. For example, <30 μm particle size is recommended for sedimentation prevention and to achieve a smooth texture.

Grinding is one of the most important unit operations when particle size reduction is required. Among various technologies, planetary ball mills are widely used that are suitable for wet and dry, fine and ultra-fine grinding of hard and brittle material due to the high mechanical stress generated (Rosenkranz *et al*., 2011). In cocoa processing, ball milling is applied in the refining stage to grind cocoa nibs or cocoa liquor into a fine paste, a process that is essential for producing high-quality chocolate with improved mouthfeel and smooth texture.

Cocoa-based beverages are valued for providing quick energy and essential nutrients, despite their high caloric input and high lipid content (Aliakbarian *et al*., 2017). However, in recent times, consumers demanded these products to be healthy and nutritionally balanced, favoring options with reduced sugar and lipidic content as well as beverages enriched with plant extracts, alternative protein sources, or probiotics (Barišić *et al*., 2023). This led to new challenges for the food industry with the need to develop newly formulated healthy cocoa-based products.

In a research and development framework, delipidated and fine cocoa powders have been tested to obtain innovative food-emulsified products stabilized via the Pickering mechanism (Joseph *et al*., 2019). Pickering emulsions are stabilized by solid particles that are irreversibly anchored at the oil–water interface (Binks and Horozov, 2006), leading to a higher stability than conventional emulsions. Moreover, fine food-grade particles contribute to enhanced sustainability and reduced toxicity, representing a valid alternative to conventional surfactants (Berton-Carabin and Schroën, 2015).

Few studies are present in the literature regarding the use of cocoa powders as pickering emulsion stabilizers, being critical aspects to consider the particle size, dispersibility and wettability in both oil and water phases, and the process conditions applied (Gould *et al*., 2013; Joseph *et al*., 2019, 2020). Wet pearl milling has been used on cocoa powders (Joseph *et al*., 2019), following the defatting step, highlighting that the cocoa butter could act as an interfering component in the stabilization of pickering emulsions. However, "dry" ball milling has not been investigated on this matrix to develop innovative pickering stabilizing agents.

Ball milling, classified as a superfine grinding technique, is gaining interest as a mechanical process that could produce superfine powders with modified physical and physicochemical properties (Gao *et al*., 2020; Meng *et al*., 2017). This could also be of interest for cocoa powders intended for the development of innovative formulated products and beverages.

Thus, this study aimed to investigate the use of ball milling (BM) on cocoa powders of two different fat content and defatted samples. The powders obtained were assessed for their physical and microstructural properties and technological functionality. Different BM process intensities were applied using two rotation rates (450 and 550 rpm) and milling times (10, 20, and 30 min). The main bioactive compounds, polyphenols, and procyanidins in cocoa powder were evaluated to understand the effects of BM on the health properties.

Material and Methods

Materials

Two commercial cocoa powders intended for chocolate beverages with different fat content (High: H; Low: L) were provided by JB Cocoa (Bangkok, Thailand). Upon arrival, the cocoa powders were packed in laminated, high-barrier plastic bags and kept in a dry and temperature-controlled room until usage (20°C).

Folin–Ciocalteu's phenol reagent (2 N) was provided by Sigma-Aldrich (St. Luis, USA); glacial acetic acid for analysis ACS, ISO was provided by PanReac AppliChem (ITW Reagents; Darmstadt, Germany); acetone and n-hexane were provided by VWR (BDH Chemicals; Fontenay-sous-Bois, France); Sodium Carbonate was provided by Honeywell Fluka (Wunstorf, Germany). Deionized water was used throughout the analysis.

Methods

Ball milling

Ball-milling (BM) treatment of cocoa powder was carried out by a planetary ball miller (Fritsch Pulverisette 6) using a jar (V = 80 ml) and balls (d = 5 mm) made of

zirconium oxide, with Ball to Powder Ratio (BPRT) 20:1 (balls weight = 74 g, sample weight = 3.6 g). In both cocoa powder samples, H and L, BM was carried out at two rotational speeds (450 and 550 rpm) at three different times (10, 20, and 30 min), that is, six energy/time combinations (here, and in the following test referred as 450/10, 450/20, 450/30, 550/10, 550/20, 550/30). To prevent excessive temperature rising during the process, the milling treatments were carried out by alternating 1 min milling followed by 1 min pause (the total duration of each treatment session were 20, 40, and 60 min for 10-, 20-, and 30-min ones, respectively). To monitor temperature changes during the treatment, the sample temperature was measured using an electronic thermometer, inserting the sensor on top of the cocoa powders within the chamber, before and just after the planned process time. Selected BM treatments were also carried out on H and L samples after defatting (DL and DH). The following treatments were carried out: 450/10, 550/10, 550/30. For each milling treatment, the yield was calculated as the ratio between the weight of the cocoa before and after milling and was expressed in %.

For each BM treatment session and cocoa powder type (ca. 3.6 g each), at least five repetitions were conducted.

Cocoa powder defatting

To evaluate the role of fat in BM treatments, cocoa powder defatting was carried out on the initial, non-treated H and L cocoa powder samples. Defatting was carried out according to the method described by Adamson *et al*. (1999). Four grams aliquot of cocoa powder was weighed using an analytical balance (Mettler Toledo B2002-S) and mixed with 25 mL hexane for 30 s at room temperature (20°C); the dispersion was then centrifuged at 4000 rpm for 10 min and the supernatant was eliminated. This procedure was repeated thrice. In the end, the cocoa powder was recovered and left to dry in a ventilated laboratory hood until the hexane residues had completely evaporated; the weight change was checked and evaporation was continued until a constant weight was achieved.

Proximate chemical composition of cocoa powders

The proximate composition of the H and L powders was carried out. Moisture was determined following the AOAC 931.04 (AOAC, 2019a), and proteins (N x 6.25) were determined following AOAC 991.20 (AOAC, 2019e). The total fat content was determined following an in-house method based on AOAC 963.15 (AOAC, 2019b), total carbohydrates of the two cocoa powders were determined following AOAC (1993, p. 8), ash content was determined following an in-house method based on AOAC 972.15 (AOAC, 2019c), and crude fiber was determined following an in-house method based on AOAC 978.10 (AOAC, 2019d).

Particle size distribution

The particle size analysis was performed using Mastersizer3000 (MS3000, Malvern Particle Size Analyzer). The non-treated (NT) and BM samples were preliminarily dispersed in deionized water at a pump speed of 2000 rpm; before testing, the samples were sonicated (2 min) to promote the breakdown of macroaggregates, and their obscuration was maintained between 3 and 6%. The particle size analysis for average particle sizes (D[3,2]: area-weighted mean diameter; SSA: Specific surface area; D[4,3] volume-weighted mean diameter; RSF: Relative span factor) was determined. The area-weighted (D[3,2]) and volume-weighted (D[4,3]) mean diameters were calculated according to Equations 1 and 2, respectively.

$$D[4,3] = \frac{\sum n_i D_i^4}{\sum n_i D_i^3} \quad (1)$$

$$D[3,2] = \frac{\sum n_i D_i^3}{\sum n_i D_i^2} \quad (2)$$

where, n_i is the frequency of occurrence of particles of class size *i* and having a mean diameter D_i.

The Relative Span Factor (RSF) was calculated according to Equation 3.

$$RSF = \frac{D_{(v,0.9)} - D_{(v,0.1)}}{D_{(v,0.5)}} \quad (3)$$

where, $D_{(v,0.5)}$, $D_{(v,0.9)}$, and $D_{(v,0.1)}$ are the particle diameters at 50, 90, and 10% volume distribution, respectively.

Thermal properties

Thermal analysis of the untreated and BM cocoa powders was carried out using a differential scanning calorimeter (DSC 8500, PerkinElmer, Waltham, MA, USA). Thermal analysis was conducted to determine the melting behavior of the fat crystals in the cocoa powders. An aliquot of the accurately weighed sample (ca. 5 mg) was placed into DSC aluminium pans (50 μL, PerkinElmer, USA) and sealed with aluminium lids. Samples were held for 2 min at −20°C, heated up to 70°C at 5°C/min, and cooled at 10°C/min to the initial temperature. Onset and peak temperatures, as well as the enthalpy variation (ΔH), were computed for every melting phenomenon observed. Moreover, the temperature interval at which the fat crystals melted was determined as the difference between the end of the melting peak observed at the highest temperature and the onset of the melting peak observed at the lowest temperature. Instrument calibration was performed using indium.

Total Polyphenol Index (TPI)

The defatted samples were analyzed (Refer Section "Cocoa powder defatting"), and TPI was carried out

on samples obtained by two different extraction procedures to determine the content of the water-soluble antioxidants (refer below) and the procyanidins fraction.

Extraction of water-soluble antioxidants

A 2 g aliquot of defatted cocoa powder was dispersed in deionized water at 70°C under constant stirring (800 rpm) on a magnetic heating plate (Intelli-Stirrer MSH-300i) for 20 min; the solids to solvent ratio was 1:8 (2 g of cocoa powder and 16 mL of water). The extract was then filtered with cellulose filters and used for the determination of TPI using the Folin–Ciocalteu method (Stratil *et al*., 2006).

Extraction of procyanidins

The extraction was performed following the method described by Di Mattia *et al.* (2013). 1 g of defatted sample was extracted with 5 mL of acetone/water/acetic acid solution (70:29.5:0.5). After vortexing for 1 min, the mixture was sonicated in an ultrasonic bath at 20°C for 10 min. Sample extracts were clarified by centrifugation (4000 rpm for 10 min) and filtered through cellulose filters; this extract was used to determine the procyanidins content using the TPI protocol.

TPI analysis

The TPI was determined according to the modified procedure by Singleton and Rossi (1965). The sample extract (0.1 mL) was diluted with deionized water to a volume of 5 mL to which 500 µL of Folin–Ciocalteu (FC) reagent was added. After 3 min, 1.5 mL of 25% Na_2CO_3 solution was added followed by deionized water and made up to 10 mL final volume. The solutions were maintained at room temperature under dark conditions for 60 min, and the total polyphenol content was determined at 765 nm using a UV-Visible spectrophotometer (P9 Double Beam UV-visible, VWR). Gallic acid standard solutions were used for calibration purposes. Results were expressed as mg gallic acid equivalents (GAE) g^{-1} DM.

Microstructure

The microstructures of the cocoa powder samples were evaluated using Confocal Laser Scanning Microscopy system (Nikon A1r, Nikon Corporation, Tokyo, Japan), controlled by the Nikon NIS Elements software version 4.40, equipped with a Plan Apochromat 60X oil (n.a. 1.40) objective and Nikon camera DS QiMc U3 (Nikon Corporation, Tokyo, Japan). Laser lines: argon 514 nm and diode 640 nm. Fluorophore: Nile Red (excitation = 488 nm; emission = 525/50 nm) and Fast Green FCF (excitation = 600 nm; emission = 640–700 nm). Detector: continuous Galvano scanner. No pretreatments were carried out on the samples before analysis.

Color

The color of the cocoa powder samples was measured by a spectrophoto-colorimeter (Konita Minolta, Chroma Meter CR-5, Tokyo, Japan) with CIE (L*a*b*). Before analysis, the instrument was calibrated using the spectral reflectance of a white calibration tile as the standard. From the L*, a*, and b* data, the Chroma (C*) and hue (h*) were calculated based on the Equations 4 and 5.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (5)$$

The samples were measured in triplicates and at room temperature (22 ± 1°C).

Dispersibility

Dispersibility was determined according to the method reported by Shittu and Lawal (2007). 3 g aliquots of each sample were dispersed in 30 mL distilled water at 27°C. The mixture was stirred contantly (800 rpm) for 1 min on a magnetic heating plate (Intelli-Stirrer MSH-300i, Gennevilliers, France) and allowed to rest for 30 min for the suspended particles to settle down before the supernatant was carefully decanted. The density of the supernatant was determined by filling an aliquot of 15 mL of the supernatant into a beaker, and the mass of the filled bottle was noted. The weight of the dispersed solid was calculated following Equation 6.

$$W_{ds} = 2(m_s - m_w) \quad (6)$$

where, W_{ds} is the weight of the dispersed solid; m_s is the mass of the supernatant; and m_w is the mass of an equal volume of distilled water. The dispersibility index (DI), expressed in percentage (%), was calculated using Equation 7.

$$DI_{(\%)} = \left(\frac{W_{ds}}{W_s}\right) \times 100 \quad (7)$$

where, W_{ds} is the weight of the dispersed solid and W_s is the weight of the initial sample.

All the weight determinations were done in triplicate using an analytical balance (0.001 g accuracy).

Statistical analysis

The significant differences between the samples were determined using analysis of variance (ANOVA).

The Tukey–Kramer test for post-hoc analysis was also used to identify the significant differences ($P < 0.05$). The statistical software StatSoft Statistica version 12.5 was also used in the analysis.

Results and Discussion

Ball milling treatments at 450 and 550 rpm for different times were carried out on cocoa powder samples with different fat contents to evaluate the effect of this mechanical process on particle size and related changes on their technofunctionality for applications in innovative chocolate-based beverages and/or other innovative formulations. These conditions, selected based on previous experiments on other complex matrices, were intended to evaluate different process intensities by using the combination of two different parameters: rotation speed (450 and 550) and duration (10, 20, 30 min) to reduce and/or modify the particle size by causing other morphological or physical changes on the cocoa powders so as to enhance the technological functionality for the development of innovative cocoa-based beverages. To unravel the role of fat in cocoa powder samples, samples at two different fat levels (H and L) were chosen, and selected BM treatments were carried out on L and H defatted samples. The effects of BM on physical properties and fat crystalline state of the powders were studied by particle size, and thermal and microstructural analyses, while the bioactive content and dispersibility were evaluated to complement the technological and health properties of BM-treated powders.

Proximate composition

The proximate composition of the two cocoa powder samples was determined to better characterize the initial systems, and the results are shown in Table 1. In general, the two products showed minor, while significant, differences for all parameters (i.e., moisture, ash, and proteins) except for the lipid fraction, as the H sample presents a fat content almost twice the L sample (16.67 and 8.95%, respectively) that confirms the initial general information provided by the company. Based on the current European legislation, both samples could be classified as "fat-reduced cocoa powders" as they contain less than 20% cocoa butter lipid content and not more than 9% water (EU, 2000). The chemical composition of cocoa powders depends, in general, on both plant varieties and processing conditions (i.e., fermentation, roasting, and alkalinization). Compositional values in terms of protein and carbohydrates of the L and H cocoa powders are in agreement with data reported in the literature with a relatively high content of carbohydrates that could be referred to products subjected to alkalinization (Razola-Díaz *et al.*, 2023). The alkalinization process could also increase the ash content, and this finding is in agreement with data reported by other authors (Adeyeye, 2016).

Table 1. Proximate chemical composition of H and L cocoa powders.

	Mean (% wet basis)	
	H	L
Moisture	6.04 ± 0.03^{b}	7.59 ± 0.01^{a}
Carbohydrates	33.76 ± 0.22^{a}	34.08 ± 0.34^{a}
Total fat	16.67 ± 0.13^{a}	8.95 ± 0.07^{b}
Protein (N x 6.25)	21.33 ± 0.19^{b}	23.85 ± 0.15^{a}
Ash	10.52 ± 0.15^{b}	12.75 ± 0.25^{a}
Crude fiber	11.68 ± 0.26^{b}	12.78 ± 0.16^{a}

Data reported are as mean ± standard deviation. $^{a–b}$Mean values followed by different letters in the same row are not significantly different at $P \leq 0.05$.

Ball milling process: Yield and final temperature

The BM yield was higher than 95% for all the samples with nonsignificant differences between the two series of samples; some losses occurred due to the difficulty in recovering the part of the powder stuck on the balls' surface and jar walls.

The temperatures of the cocoa powders before and after the BM treatment are shown in Table 2. BM was carried out in a noncontrolled temperature chamber, and an increase in the temperature due to the thermal stresses induced by the ball milling was expected. Despite the procedure (1 min milling followed by 1 min pause), all samples showed a temperature increase of different entities depending on the process parameters and sample type. In general, the increase in temperature of cocoa powders was directly related to the milling time and rotational speed, where the former parameter had a major effect in agreement with other authors (Burmeister and Kwade, 2013).

Furthermore, at the same processing conditions (rotational speed and time), no significant differences between the final process temperature of the two defatted powders (DL and DH) were observed (Table 2). Moreover, the defatted DH and DL samples had significantly higher temperature than the corresponding H and L powders, suggesting a major role of the lipid fraction in the thermal energy generated by the process. At environmental conditions, cocoa butter is mostly in a crystalline state that, depending on the thermal history could be in six different polymorphic crystalline states (I–VI) characterized

Table 2. Initial and final temperature of the BM-treated samples.

	BM treatment	Temperature (°C)	
		Low fat	High fat
	NT	24.2 ± 0.1^{k}	
Non-defatted	450/10	30.3 ± 0.1$^{g–i}$	28.4 ± 0.3^{j}
	450/20	30.9 ± 0.2$^{f–h}$	29.9 ± 0.6$^{h–j}$
	450/30	32.4 ± 0.2^{d,e}	31.4 ± 0.2$^{e–g}$
	550/10	31.7 ± 0.2$^{e–g}$	29.1 ± 0.5^{i,j}
	550/20	34.3 ± 1.1^{c}	31.8 ± 0.1$^{e–g}$
	550/30	36.1 ± 0.2^{b}	33.2 ± 0.3^{c,d}
Defatted	450/10	29.4 ± 0.6^{i,j}	32.2 ± 0.1$^{d–f}$
	550/10	34.3 ± 0.2^{c}	34.0 ± 0.4^{c}
	550/30	39.0 ± 0.1^{a}	38.7 ± 0.1^{a}

NT, non-BM, initial.
$^{a–j}$ Mean values followed by the same letters in the same column are not significantly different at a significance level of $P \leq 0.05$.

by different melting temperatures, that is, in any case, are lower than 40°C (Palmieri and Hartel, 2019). The results of this study suggest that during the process, a part of the thermal energy produced by the intense mechanical stresses is absorbed and used as latent heat to melt the fat crystals. This determines a lower temperature increase of the H and L samples than the corresponding defatted cocoa powder where, due to the lack of the lipid fraction, the thermal energy is directly converted in sensible heat and corresponding temperature increase.

Particle size analysis

Particle size data of the untreated and BM-treated powders are shown in Table 3, while in Figure 1 the particle size distributions (PSD) of the untreated and BM powders (H/L/DL/DH) at 550 rpm are shown. Both the untreated H and L powders showed a monomodal distribution of the particle size with a rather wide-sized interval spanning from less than 10 mm to over 100 mm. In both cocoa powder samples, a shoulder at the higher particle size was observed, particularly for the H samples. This trend seems to disappear in the defatted samples with a decrease of the peak and narrowing of the distribution curve in the DH sample. These results are also confirmed by the D[4.3], D[3.2], and SSA values, but in particular by the RSF (Table 3) index values that provide information about the spread or distribution of the particle sizes of a sample (Merkus and Merkus, 2009). The RSF of the defatted samples are significantly lower than that of H and L. These results could be explained by considering that during the grinding of cocoa mass to produce cocoa powder, cocoa butter tends to cover the surface of the particles determining larger particles and increasing the heterogeneity of the size distribution (Beckett, 2019). Distribution particle size curves of the defatted samples were similar to those observed by Joseph *et al*. (2019) in their study on cocoa powders processed to obtain Pickering emulsion stabilizers, associated with both micron-sized and large shapeless particles with an average size of ca. 14 mm.

In high-fat BM samples, a statistically significant increase in D[4,3] was observed at longer milling times at 450 rpm. At 550 rpm, a more pronounced size increase occurred during shorter processing times compared to longer treatments. In contrast, D[3,2] was only minimally affected. Concerning the treated L powders, a size reduction was observed in BM at intensities above 450 rpm for 10 min, accompanied by a decrease in D[3,2] and RSF, indicating that the process impacted larger particles, resulting in a smaller and more uniformed size distribution (Figure 1A). The overall effect of the BM on H and L was different, which could be ascribed to the difference in fat contents of the cocoa powder. The high energy process could cause an increase of the D[4.3] in the product with the higher lipidic fraction because of the partial melting of fat crystals, which causes particle aggregation and a consequential decrease in particle size reduction efficiency of the BM process (Burmeister and Kwade, 2013). This hypothesis is also supported by the temperature of the sample at the end of the process, that is, 550/20 and 550/30 equal to 34.3 and 36.1°C, respectively (Table 2). On the other hand, in the case of the L sample, a breakdown effect was observed due to the high mechanical energy typically associated with the BM process, for which it is generally applied.

Interesting results were observed when the defatted BM L (DL) and H (DH) samples were considered in general, except DL450/10, with an increase in size. In detail, in the case of the DH, a simultaneous increase in the RSF was observed in a majority of the cases. This means that, contrary to our expectations, the BM process conditions applied in this study do not cause a breakdown of the particles, but they determine the effects that lead to their increase. Different causes and mechanisms that are implied in this affect the mechanical stress and temperature. Particle aggregation and entrapment could occur leading to particles of larger dimensions even in the absence of fat. This could be favored by localized physical and conformational changes of the cocoa powder components (e.g., protein denaturation, sugars melting) that could induce reversible and/or irreversible interaction between the particles (Ramadhan and Foster, 2018). Joseph *et al*. (2019) by applying wet pearl milling on defatted cocoa powders found that this process leads to the formation of large particles with a more open structure than the dense, nonprocessed ones as a result of an initial disentanglement of the large primary

Table 3. Particle size properties of untreated L and H cocoa powders and BM for different times.

Cocoa powder type	BM treatment	D[3,2] µm	D[4,3] µm	SSA (m^2/g)	RSF
H	NT	11.51 ± 0.22^{f}	22.57 ± 1.53^{f,g}	0.375 ± 0.007^{i}	2.95 ± 0.24^{b}
	450/10	10.88 ± 0.04^{g}	28.52 ± 0.49^{d}	0.397 ± 0.010^{h}	2.77 ± 0.02^{b,c}
	450/20	11.52 ± 0.07^{f}	35.98 ± 2.11^{a}	0.374 ± 0.030^{i}	3.27 ± 0.13^{a}
	450/30	11.06 ± 0.10^{g}	33.88 ± 1.42^{a,b}	0.391 ± 0.030^{h}	3.17 ± 0.09^{a}
	550/10	12.68 ± 0.13^{d}	36.13 ± 2.07^{a}	0.340 ± 0.003^{j}	2.88 ± 0.10^{b,c}
	550/20	10.10 ± 0.06^{h}	24.90 ± 0.75^{e}	0.428 ± 0.002$^{e–g}$	2.34 ± 0.04^{d}
	550/30	10.18 ± 0.07^{h}	29.84 ± 1.50^{c,d}	0.425 ± 0.004^{f,g}	2.92 ± 0.08^{b,c}
DH	NT	8.31 ± 0.01^{k,l}	12.60 ± 0.11^{k}	0.547 ± 0.001^{a}	1.89 ± 0.01^{g}
	450/10	13.18 ± 0.13^{c}	27.78 ± 0.48^{d}	0.345 ± 0.003^{j}	2.71 ± 0.03^{c}
	550/10	14.22 ± 0.04^{b}	31.52 ± 0.24^{b,c}	0.320 ± 0.001^{k}	2.87 ± 0.02^{b,c}
	550/30	14.86 ± 0.05^{a}	31.02 ± 0.74^{c}	0.306 ± 0.001^{k}	2.18 ± 0.02$^{d–f}$
L	NT	11.50 ± 0.14^{f}	20.92 ± 0.69^{g,h}	0.376 ± 0.005^{i}	2.74 ± 0.12^{c}
	450/10	10.30 ± 0.20^{h}	17.72 ± 0.49^{j}	0.420 ± 0.002^{g}	2.10 ± 0.01$^{e–g}$
	450/20	7.85 ± 0.03^{m}	15.00 ± 0.38^{j,k}	0.550 ± 0.002^{a}	2.14 ± 0.04$^{d–f}$
	450/30	8.40 ± 0.03^{j,k}	15.58 ± 0.24^{i,j}	0.514 ± 0.002^{b}	2.12 ± 0.02$^{d–f}$
	550/10	7.94 ± 0.01^{l,m}	15.53 ± 0.15^{i,j}	0.544 ± 0.010^{a}	2.28 ± 0.01^{d,e}
	550/20	8.75 ± 0.10^{i,j}	17.26 ± 0.79^{i,j}	0.494 ± 0.005^{c,d}	2.13 ± 0.03$^{d–f}$
	550/30	8.84 ± 0.08^{i}	16.44 ± 0.42^{i,j}	0.489 ± 0.005^{d}	1.95 ± 0.03^{f,g}
DL	NT	10.44 ± 0.05^{h}	17.78 ± 0.25^{j}	0.435 ± 0.003^{e,f}	2.34 ± 0.03^{d}
	450/10	9.03 ± 0.02^{i}	20.34 ± 0.35^{h}	0.503 ± 0.001^{b,c}	2.74 ± 0.02^{c}
	550/10	10.32 ± 0.04^{h}	23.74 ± 0.75^{e,f}	0.441 ± 0.001^{e}	2.22 ± 0.03^{d,e}
	550/30	12.16 ± 0.05^{e}	21.40 ± 0.17$^{f–h}$	0.374 ± 0.001^{i}	2.33 ± 0.02^{d,e}

$^{a–m}$Mean values followed by the same letters in the same column are not significantly different at a significance level of $P \leq 0.05$.
D[3,2], area-weighted mean diameter; D[4,3], volume-weighted mean diameter; DH, defatted high fat; DL, defatted low fat; H, high fat; L, low fat (L); NT, non-treated; RSF, relative span factor; SSA, specific surface area.

particles, followed by their aggregation and rearrangement providing them with an open, expanded structure. The application of BM on complex food matrices like cocoa powder is very scarce and further research is required.

Microstructure

Figure 2 illustrates the microstructural properties of cocoa powder. Images were taken for both defatted and non-defatted L and H samples, before (NT) and after undergoing BM treatment for 30 min at 550 rpm. The non-treated H and L samples were characterized by round and irregularly shaped granules. In contrast, the non-treated defatted H and L samples had less defined shapes with jagged edges caused by cavities formed during the defatting process. Additionally, the surfaces of the defatted samples appeared rougher compared to their non-defatted counterparts, due to the removal of the fat layer that typically coats cocoa powder grains, giving them a smoother, more uniform surface (Do *et al.*, 2011; Jacquot *et al.*, 2016). The green and red spots visible within the granules are related to the polyphenol molecules, abundant on the particles' surface, that can be identified by their natural autofluorescence (Calva-Estrada *et al.*, 2021). The 30-min BM treatment led to the formation of large agglomerates caused by the prolonged milling operation. Although BM is typically used for size reduction, extended processing times can result in a shift from a particle size reduction phase to an increasing size phase due to the formation of agglomerates. This occurs because the high-energy mechanical shocks cause the particles to irreversibly adhere to each other (i.e., cold welding) (Fadda *et al.*, 2009). In the defatted samples, a reduction in red and green fluorescent areas was observed after BM treatment, indicating a greater degradation of the bioactive molecules located on the surface of the granules compared to non-defatted samples. The phenomenon arises from the greater intensity of the BM treatment, confirmed by the higher temperatures measured at the end of the process, when defatted samples are processed, compared to their non-defatted analogs processed under the same conditions (Table 2).

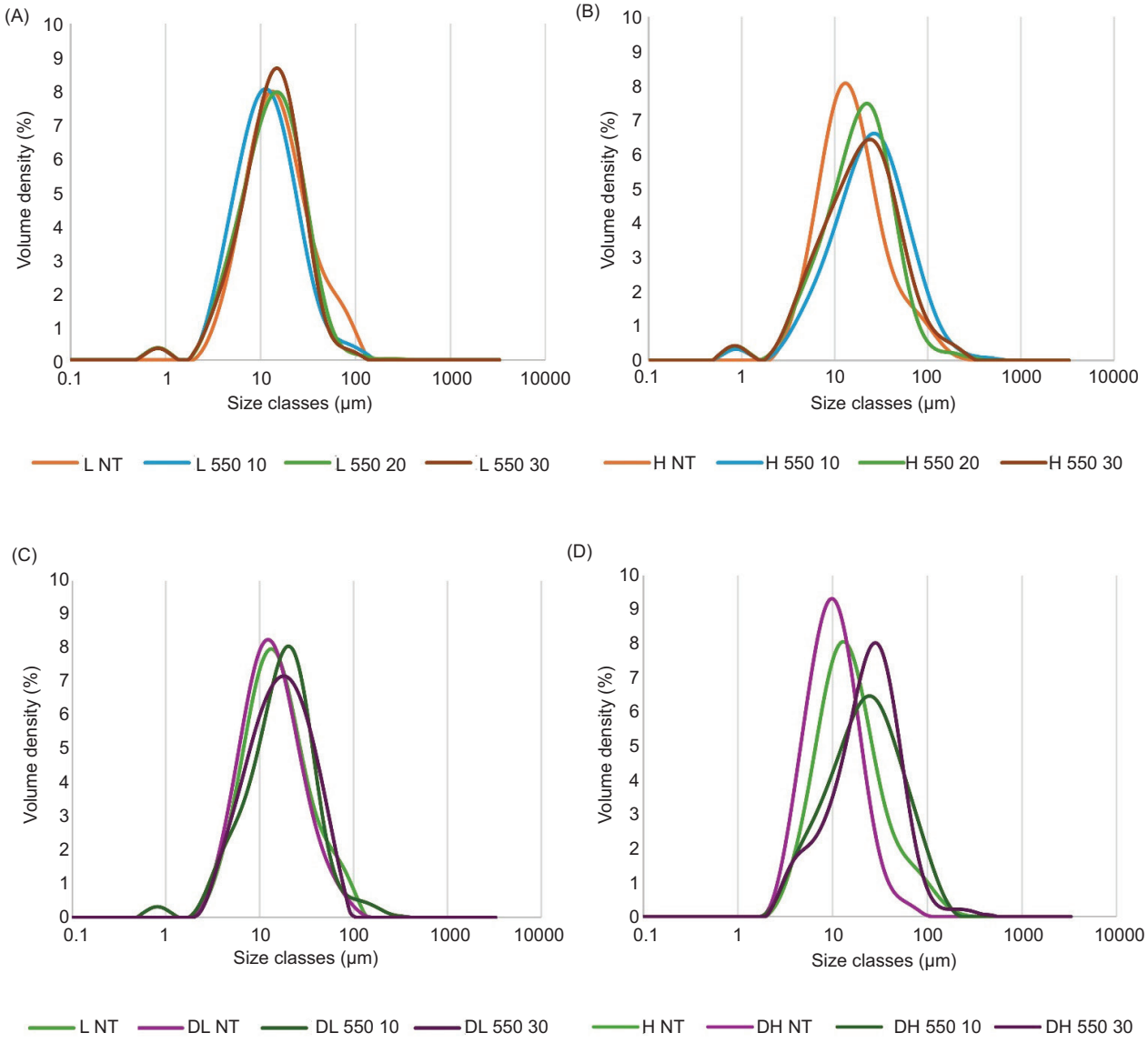

Figure 1. Particle size distribution of the L (A), H (B), DL (C), DH (D) cocoa powders BM at 550 rpm for different times. NT, non-treated; L, low fat (L); H, high fat; DL, defatted low fat; DH, defatted high fat.

Thermal properties

Thermal analysis by differential scanning calorimetry was carried out on L and H cocoa samples before and after BM treatments. The examples of the thermograms of untreated and L and H BM samples subjected to 550 at 10 and 30 min are reported in Figure 3. In general, three endothermal phenomena of different intensities were observed from 5 to 40°C depending on the type of sample and BM treatment applied. No endothermic phenomena were observed in the untreated and BM L and H defatted cocoa powder samples (data not shown), meaning that these endothermic peaks could be attributed solely to the lipid fraction and related to their corresponding melting. Cocoa butter crystallizes in six different polymorphic forms, namely γ, α, β'2, β'1, β2, and β1 with the γ being the least and β2 and β1 the most stable ones respectively (Roos and Drusch, 2015). Each polymorph, besides size and conformation, is also characterized by different melting temperatures, being equal to 17.3, 23.3, 25.5, 27.5, 33.8, and 36.3°C, respectively (Castro-Alayo *et al.*, 2022; Declerck *et al.*, 2021). Therefore, it is possible to hypothesize that the thermal events could be overall associated with the presence of two (L) or three (H) main groups of polymorphs that, in turn, are the consequences of the preliminary process and thermal conditions at which the powders have been subjected.

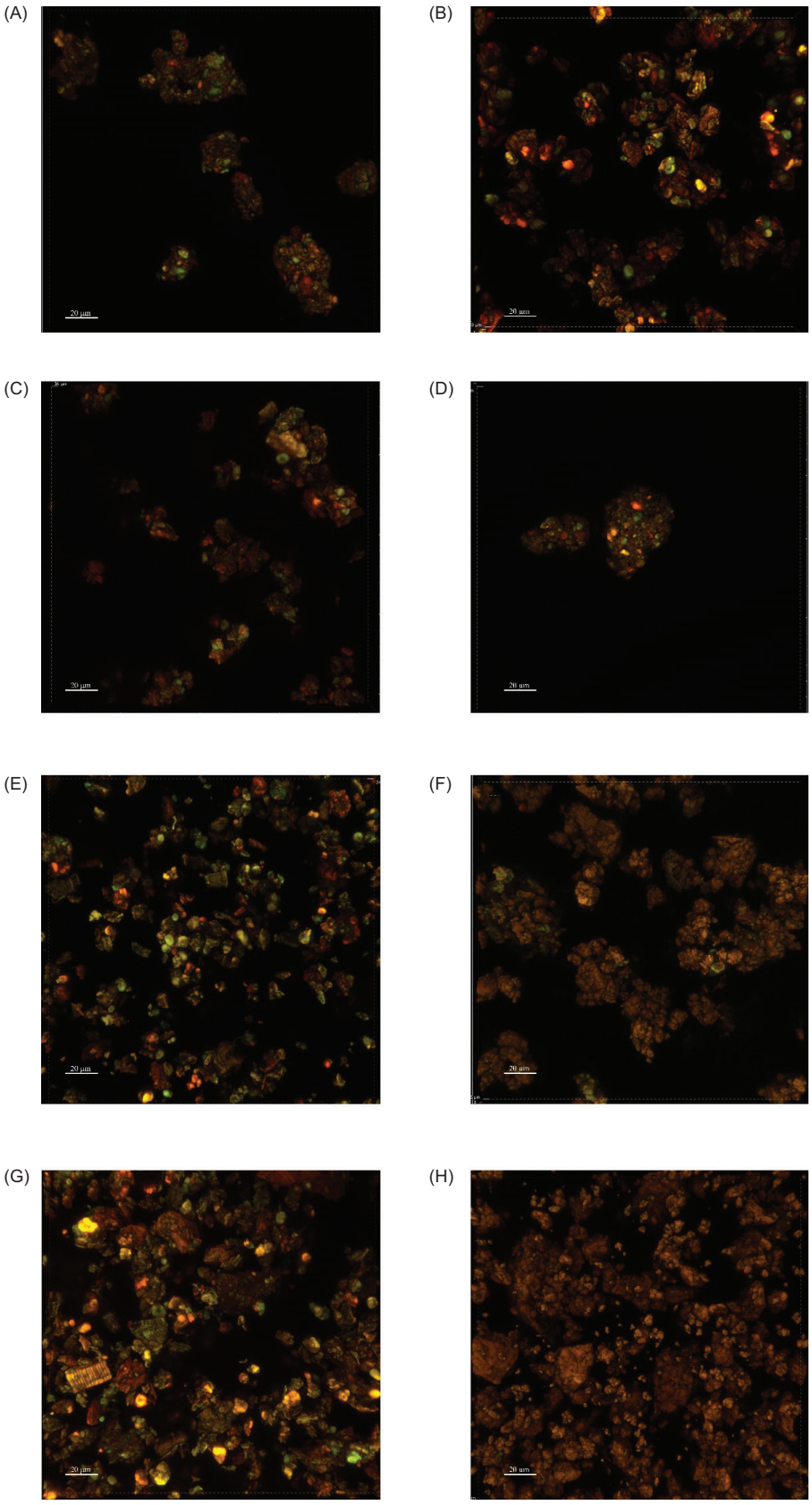

Figure 2. Confocal Scanner Laser Microscopy (CLSM) images of NT (A, C, E, G) and BM 550/30 (B, D, F, H) of H (A, B), L (C, D), DH (E, F) and DL (G, H) cocoa powder samples. DH, defatted high fat; DL, defatted low fat; H, high fat; L, low fat; NT, non-treated.

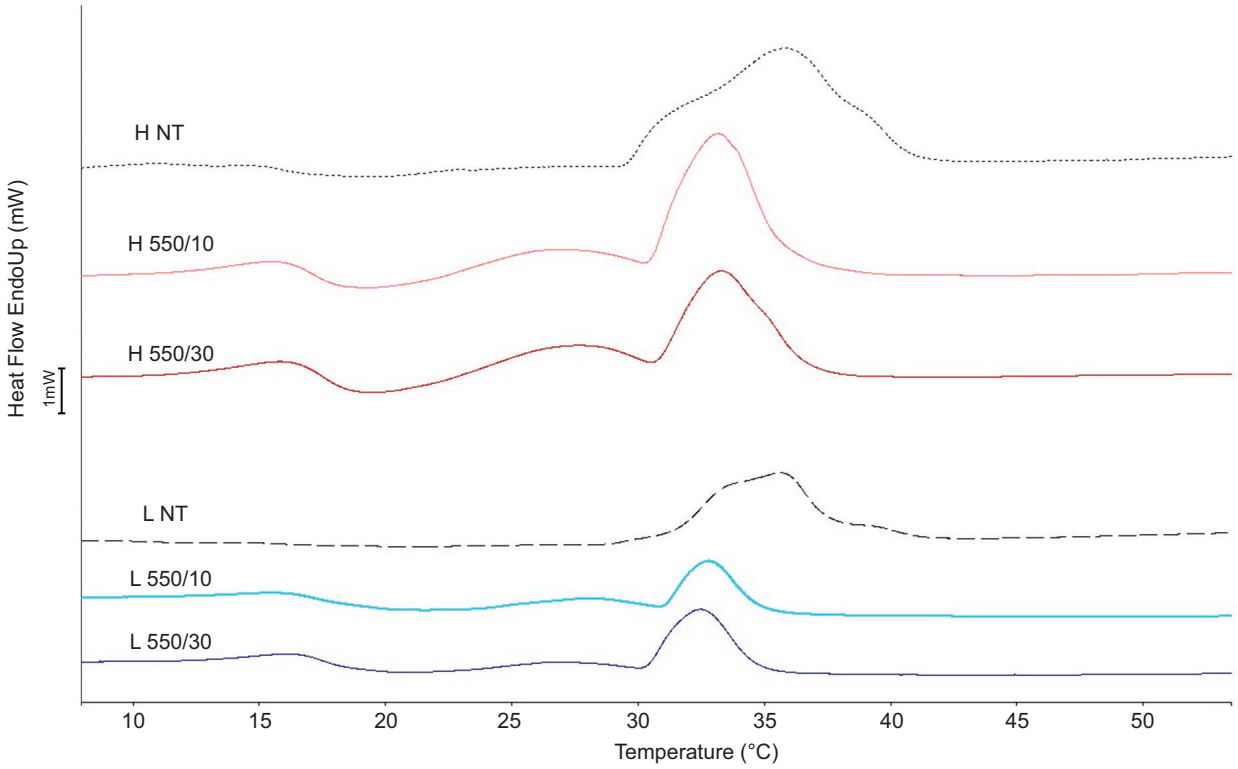

Figure 3. Differential scanning calorimetry analysis of H NT (black dotted line), H 550/10 (pink solid line), H 550/30 (red solid line), L NT (black dashed line), L 550/10 (light blue solid line) and L 550/30 (blue solid line).

Table 4. Thermal properties of untreated and BM treated high- and low-fat samples for different processing timeS at rotation of 550 rpm.

	1st peak			2nd peak			3rd peak			Melting T range (°C)
	onset (°C)	max (°C)	ΔH (J/g)	onset (°C)	max (°C)	ΔH (J/g)	onset (°C)	max (°C)	ΔH (J/g)	
H NT	5.81^{b}	10.85^{b}	2.04^{b,c}	20.29^{b}	27.57^{a}	1.81^{a}	28.75^{b}	35.64^{a}	21.06^{a}	34.80^{a}
H 550/10	7.93^{a,b}	15.85^{b}	3.35^{a,b}	21.21^{b}	27.47^{a}	6.40^{a}	30.61^{a,b}	33.37^{b}	13.86^{b}	29.67^{b}
H 550/30	7.87^{a,b}	16.00^{b}	3.91^{a}	21.41^{b}	27.63^{a}	8.20^{a}	30.60^{a,b}	33.26^{b}	11.38^{b,c}	28.91^{b}
L NT	5.14^{b}	12.01^{a,b}	0.93^{c}	n.d.	n.d.	n.d.	31.21^{a}	35.75^{a}	10.10^{c,d}	35.11^{a}
L 550/10	11.48^{a}	16.41^{b}	1.66^{c}	22.74^{a}	28.55^{a}	1.70^{b}	30.54^{a,b}	32.84^{b}	4.86^{e}	23.64^{c}
L 550/30	8.81^{a,b}	16.22^{b}	1.97^{b,c}	22.91^{a}	27.46^{a}	1.50^{b}	30.57^{a,b}	32.65^{b}	4.78^{e}	26.68^{a}

$^{a–d}$Mean values followed by different letters in the same column are significantly different at a significance level of $P \leq 0.05$.
H, high fat; L, low fat; NT, non-treated.

The thermograms of the BM highlighted some changes in the thermal behavior, both in the presence and maximum temperature of the endothermic peaks and in their intensity. In Table 4, the onset and maximum temperatures, the enthalpy variation (ΔH) of the different peaks observed, and the temperature range in which all the thermal phenomena occur are reported.

In general, both non-treated L and H cocoa samples presented a large endothermic peak at around 35°C (third peak) and a small one (first peak) at around 12°C. In both cases, the peaks were more pronounced in the H samples, proportional to the higher fat content. Furthermore, the non-treated H powder showed another endothermic phenomenon (second peak) of a very limited entity in the 20–28°C temperature

range. The larger peak at ca. 35°C occurs in a rather large temperature range, and it is characterized by the presence of "shoulders" suggesting the occurrence of overlapping melting transitions of different polymorphs. Palmieri *et al.* (2019) in their study on the crystallization of cocoa butter in cocoa powders at two different fat levels, natural or subjected to different alkalinization conditions, found similar results with the main melting peak at temperatures ca. 31,7–35°C with differences related to the different lipid content and processing conditions applied.

According to some authors (Beckett, 2009; Deora *et al.*, 2013; Marangoni and McGauley, 2003) the β2' and β1' forms are known to melt in the range of temperatures between 20 and 27°C, while the β1 and β2 between 29 and 34°C indicating the presence of these polymorphs in the cocoa powders originated during their manufacturing. As for the first peak at the lower temperature, this could be associated with the α-polymorph, and in this case, the entity of the peak is associated with the lipid content of the H and L cocoa powders. The BM treatment significantly affected the crystalline structure of cocoa butter in both H and L samples. In particular, a significant increase in the onset and the T_{peak} of the first endothermic event was observed accompanied by a progressive increase in ΔH that correlated with the BM time. Concerning the third large peak occurring at ca. 35°C, a narrowing effect of the BM treatment was noted with a decrease in the temperature range of occurrence and of the corresponding enthalpy. On the other hand, the appearance (L samples) or a significant increase (H samples) of the second peak occurred with no change in the maximum T_{peak}.

The changes in the thermal behavior of the lipidic fraction of both the L and H samples due to the ball milling process and its intensity suggest that the thermal energy induced during the high energy mechanical process may cause, depending on the temperature reached by the sample during the BM process, a solid–liquid transition of the cocoa butter polymorphs depending on their specific melting temperature followed by their recrystallization at the end of the process upon recovery from the cell. This can determine a general rearrangement of the crystalline profile of the cocoa butter of the samples. From the results obtained, we speculate that the samples subjected to the BM conditions seem to experience an effect similar to that induced by the tempering process with melting and recrystallization of the lipid fraction. However, the process should be further investigated by utilizing additional analysis techniques (i.e., X-ray diffraction) and controlling the cooling phase after milling. The change of the polymorphic pattern of the L and H BM cocoa powders could lead to diverse technological functionalities and performances in cocoa-based foods and beverages that have not been explored so far but that could be interesting to exploit for future investigations.

Color

The effect of the BM process and defatting on the color characteristics of both L and H samples have been evaluated. Table 5 shows the L*, a*, and b* values along with those of the corresponding chromatic indices Chroma (C*), hue (h*). The initial, non-BM treated L and H powders are characterized by slightly different L*, being higher in the L sample, and similar a* and b* values with corresponding minor differences of the C* and hue index values. The colorimetric properties of cocoa powders are in general due to the different preliminary process applied and in particular the alkalinisation and the roasting (Di Mattia *et al.*, 2011). Roasting conditions highly contribute to the L* value and other colorimetric indices of the cocoa products by the effect on the polymerization of polyphenols and the Maillard reaction and related production of brown-color melanoidins (Krysiak, 2006). On the other side, the alkalinization, while important for the sensory properties of the cocoa products and solubility, may cause the oxidation, polymerization and degradation of polyphenols (Andres-Lacueva *et al.*, 2008; Gu *et al.*, 2006) that cause a decrease in the L* value of the cocoa powders. Di Mattia *et al.* (2011) in a study on the technological functionalities of cocoa powders obtained by different alkalinization conditions evaluation found values of L* between 59.17 for the "natural" processed powders and ca. 25.12 for the samples subjected, based on the pH to the more intense alkalinization conditions.

BM did not affect the color properties of the L samples despite the change in size. On the contrary, a decrease of C* was observed toward a red or brown color in the H cocoa powders that increase in both series of samples at a higher process time. This could be attributed to the process-induced changes on the cocoa particles and/or their surface including the aggregation (Table 2) along with the fat polymorphism, thus changing light physical interactions (refraction, reflection, etc.) with the surface of solid particles, hence their color (Gao *et al.*, 2020).

Defatting caused a significant increase in the L* of both L and H cocoa powders, while for the latter samples, a decrease of the redness (a*) occurred that, in turn, also affected the corresponding hue angle, indicating a change toward lighter and yellow or brown color. This change could be due to the effect of both the defatting step that removed the lipids present on the surface exerting a main light-reflecting ability and to the specific coloring properties of the defatted cocoa components not evident in the presence of the lipidic fraction. However, upon BM a significant decrease of the L* value was observed in both

Table 5. Colorimetric parameters, Chroma (C*), and hue (h*) of non-treated, defatted, and corresponding BM L and H cocoa powders.

Sample type and BM treatment	L*	C*	h*
H NT	34.47 ± 0.13^{h,i}	23.55 ± 0.03$^{a–c}$	53.52 ± 0.05^{e}
H 450/10	34.43 ± 0.01^{h,i}	17.99 ± 0.01^{k}	55.47 ± 0.11^{b,c}
H 450/20	34.69 ± 0.11^{h}	16.25 ± 0.32^{l}	53.98 ± 0.05^{e}
H 450/30	33.56 ± 0.88^{i}	15.37 ± 0.07^{m}	51.78 ± 0.08^{h}
H 550/10	36.50 ± 0.20$^{d–g}$	18.39 ± 0.36^{k}	55.05 ± 0.18^{c,d}
H 550/20	33.55 ± 0.45^{i}	15.44 ± 0.28^{m}	52.52 ± 0.26^{f}
H 550/30	27.80 ± 0.07^{l}	16.21 ± 0.23^{l}	51.45 ± 0.21^{h}
DH NT	45.65 ± 0.10^{a}	21.64 ± 0.04^{g,h}	57.69 ± 0.05^{a}
DH 450/10	38.47 ± 0.34^{c}	22.19 ± 0.49^{f,g}	54.64 ± 0.39^{d}
DH 550/10	36.24 ± 0.01^{f,g}	21.48 ± 0.03^{h,i}	55.73 ± 0.10^{b}
DH 550/30	31.25 ± 0.01^{k}	20.56 ± 0.02^{j}	53.95 ± 0.02^{e}
L NT	37.41 ± 0.35^{d,e}	23.72 ± 0.04^{a}	51.92 ± 0.06^{g,h}
L 450/10	35.83 ± 0.04^{g}	22.40 ± 0.14^{e,f}	51.70 ± 0.04^{h}
L 450/20	36.61 ± 0.19$^{d–g}$	22.34 ± 0.07^{e,f}	52.47 ± 0.11^{f,g}
L 450/30	37.49 ± 0.19^{c,d}	23.10 ± 0.05$^{a–d}$	52.55 ± 0.13^{f}
L 550/10	37.28 ± 0.01^{de}	22.45 ± 0.21$^{d–f}$	52.60 ± 0.07^{f}
L 550/20	36.45 ± 0.21$^{e–g}$	22.61 ± 0.12$^{d–f}$	52.55 ± 0.03^{f}
L 550/30	37.00 ± 0.24$^{d–f}$	23.01 ± 0.08$^{b–e}$	52.65 ± 0.03^{f}
DL NT	40.08 ± 0.06^{b}	23.63 ± 0.14^{a,b}	53.61 ± 0.01^{e}
DL 450/10	36.13 ± 0.04^{f,g}	22.94 ± 0.04$^{c–e}$	51.59 ± 0.02^{h}
DL 550/10	32.28 ± 0.07^{j}	20.92 ± 0.05^{i,j}	50.66 ± 0.20^{i}
DL 550/30	23.91 ± 0.02 ^{m}	6.98 ± 0.01^{n}	46.03 ± 0.27^{l}

$^{a–n}$Mean values followed by the same letters in the same column are not significantly different at a significance level of $P \leq 0.05$.
DH, defatted high fat; DL, defatted low fat; H, high fat; L, low fat (L); NT, non-treated.

Table 6. Dispersibility of selected cocoa powders.

Cocoa powder type	BM treatment	ds W (g/100g)	DI (%)
H	NT	1.19 ± 0.07^{f}	11.58 ± 0.73^{f}
	450/10	1.60 ± 0.08^{e}	15.58 ± 0.81^{e}
	550/10	0.62 ± 0.05^{g}	6.06 ± 0.53^{g}
DH	NT	1.98 ± 0.04^{c}	20.96 ± 0.56^{c}
	550/10	2.75 ± 0.05^{a}	26.84 ± 0.53^{a}
L	NT	1.66 ± 0.08^{d,e}	16.22 ± 0.81^{d,e}
	450/10	1.18 ± 0.08^{f}	11.47 ± 0.81^{f}
	550/10	1.91 ± 0.08^{c,d}	18.61 ± 0.81^{c,d}
DL	NT	2.15 ± 0.06^{c}	19.27 ± 0.40^{c}
	550/10	2.44 ± 0.08^{b}	23.81 ± 0.81^{b}

DI, dispersibility index; ds W, dispersed solid weight.
$^{a–g}$Mean values followed by the same letters in the same column are not significantly different at a significance level of $P \leq 0.05$.

H and L values indicating a lower lightness likely related to the agglomeration and lower reflectance ability of the corresponding cocoa particles.

Dispersibility

With the main scope to evaluate the applicability of BM cocoa particles to produce innovative cocoa-based beverages, the dispersibility in aqueous systems has been evaluated and the results of the Dispersibility Index (DI) of selected BM L and H (450 and 550, 10 min) and corresponding defatted powders are reported in Table 6. The DI was used to evaluate the dispersion efficiency in liquid systems as required in many fields (Galet *et al.*, 2004).

In H and L powders, both the fat content and the entity of the BM process, along with the corresponding effects on the particle size and the physical and surface properties of the particles affect the DI. However, it is not possible to identify, at the same BM conditions and/or fat content, the main criteria affecting the affinity of the cocoa powders for an aqueous matrix, except for the non-treated L and H powders where the lower initial fat content is the main factor related to a higher DI of the former product. On the other side, at the same BM time, the BM rotation rate causes a different effect on the DI even if both samples show a decrease in the particle size, a factor that should increase the dispersibility with an increase in the surface. For the 450/10 treated samples, higher and lower DI values were observed than the corresponding non-treated, for H and L, respectively. On the other hand, at 550/10, the effects seem to be more related to the particle size as a main decrease of the DI in H was observed related to the BM-induced agglomeration, while an opposite effect occurred in the L powders where the same treatment induced a decrease in the particle size.

Defatting determines a significant increase of the DI of both H and L to very similar values highlighting the main role of the lipid fraction in limiting the dispersibility of the cocoa particles. The BM 550/10 DH and DL samples, independent of the changes in the particle size (Table 3), showed a further and significant increase of DI, higher in the defatted H sample.

The dispersibility of cocoa powders depends on several factors being affected by the specific affinity of the particles for the dispersing agent along with the forces acting between the primary particles and between particles and liquid (Galet *et al.*, 2004). Additional environmental conditions such as agitation and temperature are critical

parameters, the first creating a water–cocoa interface and the second decreasing the cohesion induced by water (Galet *et al*., 2004).

Based on these results, it could be hypothesized that independent of the size of the particles, the BM process and its intensity could cause particle surface changes that may under certain conditions cause the exposition of more hydrophilic groups of the proteins and complex carbohydrates that increase the interactions with water molecules (Joseph *et al*., 2019, 2020). Breaking down, aggregation and rearrangement of the particles in the larger ones could also generate microporosity similar to that of an agglomeration process that could contribute to their dispersion in aqueous systems. On the other hand, in the L and H samples, the high energy process, which also promotes powder aggregation, may cause lipids to transfer onto the surface. This effect is further enhanced by the increase in temperature, which could trigger a solid-to-liquid transition, resulting in liquid fat forming a hydrophobic layer around the larger particles.

Total Polyphenol Index

The effect of BM treatment on the TPI and procyanidin content of the differently processed L and H cocoa powders was investigated and results are reported in Table 7. The BM treatment significantly affected the TPI, with an effect that varies based on the processing conditions and fat content of the samples. In H samples, a decrease in TPI was observed at both rotational speeds tested, with a positive correlation to the duration of the ball milling process. In contrast, for L samples, a significant decrease in TPI was noted only for BM treatments higher than 10 min, although no clear correlation between rotational speed and milling time could be found. In defatted samples, a 10-min BM treatment was sufficient to cause a significant reduction in polyphenol content at both rotational speeds with an even greater effect than the 30 min processing time. The same trend was observed in L powders processed under the same conditions, suggesting a correlation between the phenomenon and the reduced content of fat of the samples.

The main classes of phenolic compounds in chocolate are flavanols, anthocyanins, and proanthocyanidins, with the latter making up more than 50% of the phenolic fraction. (Hii *et al*., 2009). However, the polyphenol content in foods, and consequently their antioxidant activity, is highly linked to the process conditions. Fermentation, drying, and roasting may either enhance or reduce polyphenol content. This is due to molecular degradation because of high temperatures or the formation of new bioactive compounds (e.g., the Maillard reaction during roasting), which are both directly influenced by processing variables such as temperature, time, and relative humidity (Di Mattia *et al*., 2013, 2017; Sacchetti *et al*., 2009).

Table 7. Total Polyphenol Index and procyanidins content of non-treated, defatted, and corresponding BM L and H cocoa powders.

Cocoa powder type	BM treatment	TPI (GAE mg/g DW)	Procyanidins (GAE mg/g DW)
H	NT	36.11 ± 2.58$^{a–d}$	25.37 ± 2.24^{a,b}
	450/10	32.23 ± 0.59$^{b–e}$	24.85 ± 1.06^{a,b}
	450/20	25.59 ± 2.62^{f,g}	22.05 ± 2.98^{b,c}
	450/30	26.84 ± 2.31$^{e–g}$	23.71 ± 3.12$^{a–c}$
	550/10	38.20 ± 1.18^{a}	21.71 ± 2.86^{b,c}
	550/20	32.04 ± 3.11$^{b–e}$	23.19 ± 1.08$^{a–c}$
	550/30	33.39 ± 3.79$^{a–d}$	22.74 ± 2.86$^{a–c}$
DH	NT	35.81 ± 2.17$^{a–d}$	25.07 ± 1.94^{a,b}
	450/10	30.40 ± 0.44$^{d–f}$	23.48 ± 1.79$^{a–c}$
	550/10	23.12 ± 2.57^{g}	27.08 ± 3.45^{a}
	550/30	31.17 ± 2.58$^{c–f}$	21.25 ± 2.32$^{b–d}$
L	NT	38.38 ± 1.29^{a}	24.86 ± 2.22^{a,b}
	450/10	38.74 ± 4.15^{a}	19.52 ± 1.95$^{c–e}$
	450/20	37.18 ± 2.26^{a,b}	20.62 ± 2.16$^{b–e}$
	450/30	22.40 ± 2.96^{g}	20.39 ± 2.56$^{b–e}$
	550/10	35.71 ± 4.83$^{a–d}$	20.50 ± 1.23$^{b–e}$
	550/20	27.23 ± 0.96$^{e–g}$	19.34 ± 2.42$^{c–e}$
	550/30	34.63 ± 4.09$^{a–d}$	19.40 ± 1.28$^{c–e}$
DL	NT	38.08 ± 1.12^{a}	24.56 ± 1.81^{a,b}
	450/10	27.41 ± 0.48$^{e–g}$	22.36 ± 0.81$^{a–c}$
	550/10	25.72 ± 1.26^{f,g}	16.67 ± 1.74^{d,e}
	550/30	36.59 ± 1.36$^{a–c}$	15.74 ± 1.01^{e}

$^{a–g}$Mean values followed by the same letters in the same column are not significantly different at a significance level of $P \leq 0.05$.
DH, defatted high fat; DL, defatted low fat; H, high fat; L, low fat (L); NT, non-treated.

The phenolic content measured in the non-treated samples was in the same range of data of commercial cocoa powders already reported in the literature (Miller *et al*., 2008). The decrease of the TPI observed in treated samples is ascribable to the high energy involved in the ball milling process where, almost the total of the energy involved in the process is dissipated as heat due to collisions and friction forces rather than for particle size reduction purposes (McCabe *et al*., 1993). The high temperatures originating during the BM treatment are expected to have a detrimental effect on the stability of bioactive compounds, such as polyphenols, significantly reducing their functionalities (i.e., antioxidant ability) (Hu *et al*., 2012; Ramachandraiah *et al*., 2016).

The smaller decrease of TPI in the defatted and L samples treated for 30 min at 550 rpm suggests that new phenomena may have been triggered by the most intense BM treatment tested. Although no reduction in particle size was noted in the samples, molecular modifications could have occurred, potentially affecting physical properties such as solubility and thus extractability, which may explain the higher TPI content measured in comparison to samples treated for a shorter time (Ramachandraiah *et al*., 2016; Wang *et al*., 2016).

The procyanidin content remained unaffected by BM treatment in H samples, but a limited decrease in L and defatted samples occurred, confirming the critical role of fat in the processing outcome. Decreases in procyanidin content were primarily observed with longer BM treatments, highlighting the detrimental effect of elevated temperatures on the stability of these molecules (Ioannone *et al*., 2015).

Conclusions

The ball milling process conditions applied in this study on cocoa powder at different fat contents and/or defatted determined changes of various nature and different entities depending on the process intensity (i.e., rotation rate and time) and the presence (or not) of the lipidic phase. Fat content had a major effect on the size reduction and PSD leading to a decrease only in the L powders, while in H the presence of fat able to absorb part of the thermal energy originating from the process led to particle aggregation and a lower temperature measured at the end of the process. The thermo-mechanical effect of the BM also affected the polymorphism of cocoa butter whose properties in the development of cocoa-based products and beverages must be investigated. The changes in the size and physical and structural properties of cocoa powder also affected the dispersibility that, depending on the fat content resulted in either enhanced or reduced. A general reduction in the biomolecule content was observed in samples after the BM treatment despite no direct correlation between the BM processing conditions and powders functionality could be determined. The results of this study indicate that BM represents an interesting technology for size reduction purposes as it could highlight the ability to induce changes in the physical and structural properties of the cocoa powders that, in turn, may affect the techno-functionality of the products. Further research is needed to deepen the effects of BM on cocoa powder at different lipidic content and to modulate the treatment conditions to tailor the effects based on the desired changes (e.g., particle size). This could allow to preserve or improve the health and technological properties of cocoa powders for the development of food ingredients and the formulation of innovative and sustainable emulsion-based food products and beverages.

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Authors Contribution

Conceptualization: P.P., S.S.; formal analysis: M.A., M.F.; data processing and visualization: M.A., M.F.; writing - original draft: M.A.; writing – review and editing: all authors; funding acquisition: P.P., S.S.; supervision: P.P., S.S.

Conflict of Interest

None.

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