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ORIGINAL PAPER

Effects of different alkali mixtures on physicochemical, microstructural, and powder properties of alkalized cocoa

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Abstract

Alkalization changes the physicochemical, bioactive, and powder flow properties of cocoa powders, so it is an essential factor affecting the quality of cocoa products. This study investigated the relationship of the alkalization process with physicochemical properties such as color, pH, and bioactive properties such as total phenolic, flavonoid, and antioxidant capacity of cocoa powders. For this purpose, natural cocoa powders were alkalized using sodium hydroxide (NaOH) or potassium carbonate (K_2CO_3), or potassium hydroxide (KOH) solutions at their different concentrations. The browning index, darkness, and pH values increased while the total phenolic content, flavonoid, and antioxidant capacity decreased depending on the type of alkali used. FTIR spectra revealed changes in the stretching vibrations in the molecular structure of alkalized cocoa powders. Even though the alkalization process did not affect the microstructure of cocoa particles visibly, treatment with different alkali agents altered the thermal behavior, compressibility and caused a decrease in the powder flowability.

Keywords Cocoa · Alkalization · Powder flowability · Bulk density

Introduction

The natural cocoa cake is derived from cocoa beans after separating cocoa butter. They are further processed (i.e., milling and alkalization) to produce cocoa powder. Cocoa powders can be classified as natural (pH 5–6), light alkalized (pH 6-7.2), medium (pH 7.2–7.6), or strong alkalized ($pH > 7.6$) [1]. It has been reported that several alkali agents such as NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2C0_3, $KHCO_3$, $(NaH_4)_2CO_3$, $Ca(OH)_2$, and $CaCO_3$ have been used for the alkalization of cocoa powder. Codex Alimentarious has approved these alkali agents as food additives (acidity regulators), indicating maximum doses in food products [2]. They can be used alone or as combinations, with different concentrations (from 1 to 6%, w/w, based on cocoa powder weight) to confer the desired color and flavor. The type of alkali and different combinations of them may show different effects on some characteristics of cocoa, such as the color, the pH, the solubility, the taste, and alkali off-flavor [3]. While using K_2CO_3 yields cocoa powders having light and red colors without affecting the taste, NaOH (especially in combination with ammonium salts) is the most widely employed alkali agent to obtain darken cocoa powders [4]. It has been reported that potassium salts yield red color, and combining potassium and ammonium compounds result in intense black colors [5]. Since the temperature plays a vital role in color formation, increasing the alkalization temperature (ranges from 60 °C to 130 °C) results in darker colors in a shorter period. Lower temperatures generally yield light and red colors, while higher temperatures give darker colors. The maximum temperature recommended to obtain dark black cocoa is reported as 135 °C, as higher temperatures adversely affect the flavor [5]. Increasing alkalization temperatures (75 °C to 82 °C) yield darken cocoa powders, while much higher temperatures (85 °C to 90 °C) may result in the loss of red chromophores and the formation of other

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color compounds [6]. Also, alkali treatment duration (i.e., 60 to 180 min for black color formation) affects color development and off-flavor generation [5]. On the other hand, depending on the alkalization process, alkali types, and temperature, the polyphenol content and antioxidant capacity of cocoa are significantly affected, and these are essential parameters that determine the cocoa quality [7]. It was reported that the increasing degree of alkalization (increase in temperature and alkali concentration) caused decreasing in bioactive compounds such as total polyphenols [8, 9].

Particle and powder properties (i.e., particle size, particle surface characteristics, and powder flow) are important parameters in terms of industrial applications (during handling, storage, and processing) [10] and might be affected by the alkalization processes applied. Until now, no study has been found in the literature reporting the effect of the alkalization process on powder properties and its relationship with the physicochemical and bioactive properties of cocoa powders.

This study aimed to investigate the effect of alkalization and the use of different alkali agents on the functional, microstructural, particle and flow properties of cocoa powders.

Material and method

Materials and chemicals

Natural cocoa powders (fat content is around 10–12%, w/w) without alkalization were provided from local market. Analytical grade Sodium hydroxide (NaOH), Potassium hydroxide (KOH), Potassium carbonate (K_2CO_3), and other chemicals were purchased from Sigma-Aldrich Co. (Steinheim, Germany).

Methods

Alkalization of cocoa powders

Natural cocoa powders were alkalized using sodium hydroxide (NaOH) or potassium carbonate (K_2CO_3), or potassium hydroxide (KOH) solutions at their different concentrations to evaluate their effects on the physicochemical properties of cocoa powders [1, 9]. In addition, a control sample with no alkalization was also prepared. The sample codes for cocoa powder samples treated with different alkalization conditions are given in Table 1. To get al.kalized cocoa powders, alkali solutions at varying concentrations and D-glucose (1%, w/w, based on cocoa powder mass) were added to cocoa powders and mixed at 75 °C for 50 min [11]. Previous studies suggest the percentages of alkali chemicals to adjust the alkalization intensity (from weak to strong) as from 1 to 6% (w/w) [4, 11–13]. Therefore, the respective percentages of chemicals for alkalization as given in Table 1 were chosen based on the findings of previous studies. The alkalized cocoa powder was dried in an oven at 70 °C for 12 h before passing through a 60-mesh sieve [8].

Table 1 Concentrations of the alkali chemicals

Sample	Alkali (%, w/w, based on cocoa powder weight)	Alkali agent
S1	2 + 2	NaOH + KOH
S2	2.5 + 2.5	NaOH + K_2CO_3
S3	6	K_2CO_3
S4	1	NaOH
SC	-	-

Physicochemical analyses

The moisture content of cocoa samples was calculated according to loss in weight in the oven method (AOAC 931.04) [14]. The powder sample was dried at around 102 °C until constant weight in the aluminum dish. It was reported as the percentage of mass lost [15].

The extractable pH was measured using the method reported by Sacchetti et al. [16] and Miller et al. [1] with slight modifications. 5 g of cocoa powder was suspended in 45 mL of distilled water at room temperature by stirring with a spoon. Then the pH was measured using a pH meter (HI2020, Hanna Instrument) calibrated at pH 4 and 10 prior to measurement. The Miller's scale [1] was used to classify samples based on pH values. Cocoa samples were considered natural, lightly alkalized, moderately alkalized, and strongly alkalized, with pH values between 5 and 6, 6-7.2, 7.2–7.6, and over 7.6, respectively [3].

The three scales of color properties (L^*, a^*, and b^*) of the samples were analyzed using a colorimeter (CM-5, Konica Minolta, Tokyo, Japan) with an observation angle of 10° and a D65 light source [17]. For this purpose, plastic petri dishes (8 cm diameter) were filled with cocoa powder and were tightly closed to prevent air gaps. The color difference with the non-alkalized (natural) cocoa powder (ΔE) as the control was calculated as follows [9]:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

Effect of alkalization process on bioactive properties of cocoa powders

Extraction procedure The method given by [9] was used with slight modifications. Cocoa powder samples were mixed with hexane at a ratio of 1:5 (w/v) for 30 min at room temperature and washed 2 times to remove the oil content and then dried at room temperature for 48 h. Defatted cocoa samples were mixed with extraction solvent (methanol: water: acetic acid 80:19:1 v/v/v), and the mixtures were kept overnight on a mechanical shaker in a dark environment at room temperature and then centrifugated at 6500 rpm for 10 min. The supernatants were collected and filtered using a 0.45 µm filter for further analysis.

Total phenolic content (TPC) The total phenolic content of the samples was determined according to the method described by Singleton and Rossi [18] with some modifications. 2.5 mL of Folin Ciocelteau's reagent (0.2 N) and 2 mL of Na_2CO_3 (7.5% w/v) solution were mixed with 0.5 mL of sample, and the mixture was incubated at room temperature for 30 min prior to centrifugation at 4500 rpm. Then, the absorbance of the sample was measured at 760 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The result was expressed as mg gallic acid equivalent (mg GAE).

Determination of antioxidant capacity The antioxidant capacity of the samples was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the copper-reducing antioxidant capacity (CUPRAC) methods described by Kayacan et al. [19]with some modifications. In the DPPH analysis, 100 µL of the cocoa extract was mixed with 4.9 mL DPPH solution. The mixture was kept in the dark for 20 min at room temperature, and the absorbance of the samples was measured at 517 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). In CUPRAC analysis, 100 µL of the sample was mixed with 1 mL solution of $CuCl_2$ (170.48 mg $CuCl_2.2H_2O$/100 mL water), 1 mL solution of Nc (0.156 g Nc/100 mL ethanol), and 1 mL solution of NH_4Ac (7.708 g NH_4Ac/100 mL water) and 1 mL distilled water. The mixture was held at room temperature for 60 min, and the absorbance of the sample was measured at 450 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The result was expressed as mg Trolox equivalent antioxidant capacity (TEAC).

Total flavonoid content (TFC) The TFCs of the samples were determined based on the method described by Zhishen, Mengcheng and Jianming [20]. First, 1 mL of the extract was incorporated with 4 mL distilled water, 0.3 mL of $NaNO_2$ (5% w/v), and, after 5 min, 0.3 mL of $AlCl_3$ (10% w/v), respectively. After the incubation at ambient conditions for 6 min, 2 mL of 1 mol/L NaOH solution was added to the mixture, and the final volume was adjusted to 10 mL with distilled water. Then, the absorbance of the mixture was measured at 510 nm using a spectrophotometer (Shimadzu UV-1800, Japan). Total flavonoid content was expressed as catechin equivalent.

Browning index

The browning index of the cocoa samples was examined using the method described by Alasti, Asefi, Maleki and SeiiedlouHeris [7] with some modifications. Briefly, 2 g of cocoa powder was mixed with 50 mL of HCl/methanol (12 M HCl, 1 mL/L) solution and held on a mechanic shaker at 25 ºC for 45 min. Then, extracts were centrifuged at 6000 rpm for 10 min, and the supernatants were filtered using Whatman 541 filter paper. The filtrates were mixed with HCl/methanol solution at a ratio of 1:1 (v/v). The absorbance (OD) was read at 460 and 525 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The browning index was calculated using the ratio (OD_{460}/OD_{525}).

Analysis of powder properties

Particle size distribution The color and application performance of cocoa powder in chocolate and cocoa-related products are affected by particle size. A laser diffraction particle size analyzer (Malvern Mastersizer) was employed to evaluate the particle size distribution of the cocoa powder samples and d_{90} values (the particle size where 90% of the volume of particles is less than the given values in microns) were determined [21].

Thermal characterization by DSC A Differential Scanning Calorimeter (DSC) (DSC-60, Schimadzu, Japan) was employed to identify the thermal properties (i.e., phase transitions and decomposition temperature) of cocoa powders. The temperature was increased to 250 ºC with a heating rate of 10 ºC/min. The decomposition peak temperature was determined where the maximum peak height of the exothermic decomposition appeared. The measurement was done once per sample [17].

Morphology characterization by SEM A Scanning Electron Microscope (SEM) was used to investigate the surface and microstructural characteristics of cocoa powders. For this purpose, a small amount of powder material was deposited on an adhesive carbon tape-covered aluminum stub and covered with gold. SEM images were captured at a 3–4 mm distance by applying an accelerating voltage of 1.5 kV [17].

Molecular characterization by FTIR analysis The chemical properties of the cocoa powders were investigated by Fourier-transform infrared spectroscopy (FTIR) (IRTracer-100, Schimadzu, Japan) coupled with an ATR accessory with a diamond cell. The spectra were recorded in the 4000–500 cm^{-1} region with 16 scans per spectrum and a resolution of 2 cm^{-1}. The background spectrum was obtained against the air [15, 22].

Powder flow analysis by PFT method

A powder flow tester (PFT610) (Brookfield Engineering Inc, UK) was used to evaluate the flow properties of alkalized cocoa powder samples [23]. PFT consisted of a shear cell (263 cm^3) and a vane lid to generate the flow function, a plot of the unconfined failure strength versus the major principal consolidation stress. The flow functions were obtained by running the standard compressibility test and standard flow function test program (Powder Flow Pro V1.2) that measures the flow properties over five major principal consolidation stresses (ranging from 0.6 to 10 kPa) in a geometric progression. The torsional and axial speeds were set at 1.0 rev h^{-1} and 1.0 mm s^{-1}, respectively [24].

Statistical analysis

ANOVA one-way comparison test and Tukey's test were performed to analyze the differences between the mean values, and the differences were considered statistically significant at a p-value less than 0.05.

Results and discussion

Influence of alkalization on physicochemical properties

The darkening of cocoa powder was affected by the type and concentration of alkali chemicals. The darker color in alkalized cocoa powder is generally more appreciated due to the intense cocoa flavors released in food products. The darkening of cocoa powders resulted from forming high molecular weight dark compounds by oxidation [7, 9]. The images of alkalized cocoa powders and their methanolic extracts are given in Fig. 1.

Table 2 exhibited moisture content, pH, L^*, a^*, and b^* color intensities, and d_{90} values of non-alkalized (natural) and alkalized cocoa powders using varied alkali combinations and concentrations under a constant temperature of 75 °C for 50 min. The amount of alkali used and the combination of different alkali agents significantly influenced the final pH values and color attributes. As can be seen from Table 2 that the alkalization increased the pH (above

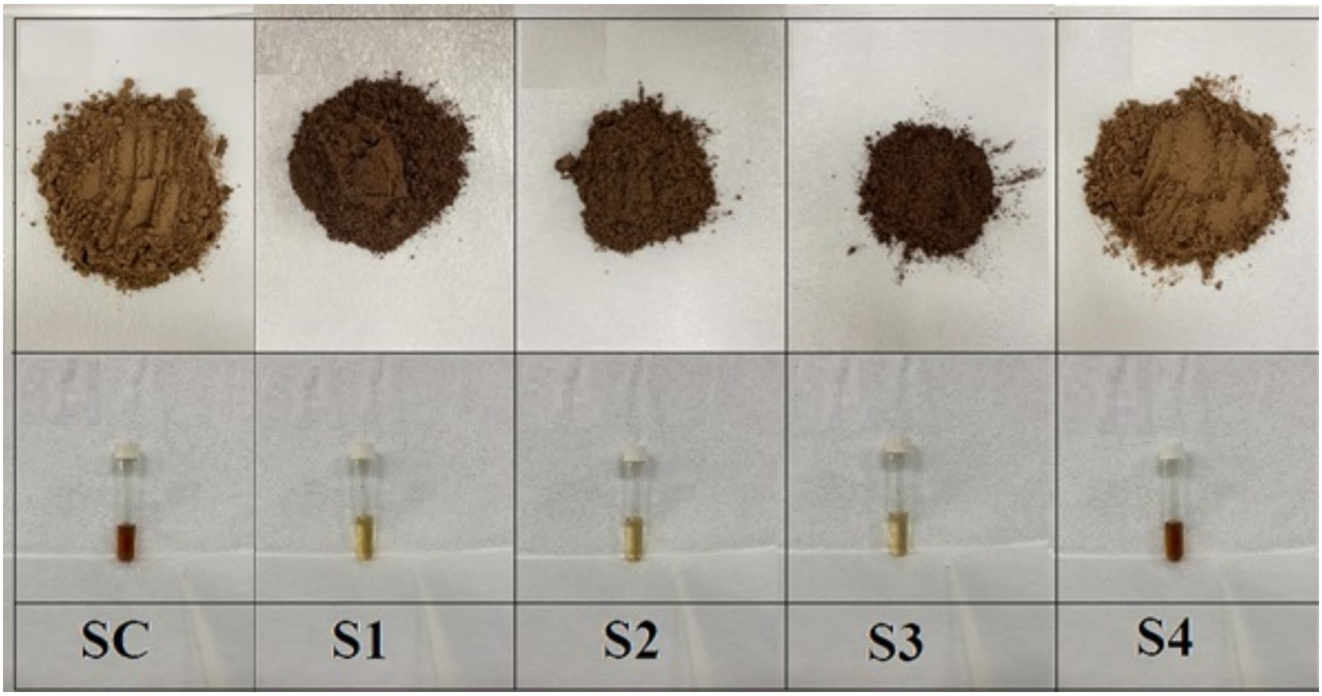


Fig. 1 Alkalized cocoa powders and their methanolic extracts. SC (natural), S1 (4% NaOH + KOH), S2 (5% NaOH + K_2CO_3), S3 (6% K_2CO_3), S4 (1% NaOH)

Table 2 Physicochemical properties of the cocoa powders

Samples	Moisture (%)	pH	L*	a*	b*	a*/b*	ΔE	d_{90}
S1	1.93	8.80 ± 0.18	26.90 ± 0.31	10.90 ± 0.42	12.00 ± 0.24	0.91 ± 0.06	23.29 ± 0.32	75.38
S2	1.64	7.91 ± 0.30	28.76 ± 0.12	12.24 ± 0.25	14.36 ± 0.10	0.85 ± 0.03	20.26 ± 0.15	67.20
S3	1.20	8.73 ± 0.22	30.71 ± 0.30	13.03 ± 0.32	17.84 ± 0.12	0.73 ± 0.04	16.71 ± 0.24	62.02
S4	1.14	5.42 ± 0.12	42.52 ± 0.62	13.49 ± 0.26	23.75 ± 0.32	0.57 ± 0.05	3.58 ± 0.40	54.64
SC	1.46	5.41 ± 0.10	45.35 ± 0.42	14.25 ± 0.33	25.81 ± 0.14	0.55 ± 0.04	0.00	74.92

Lightness L* (from white = 100 to black = 0), a* (from red = + to green = -), and b* (from yellow = + to blue = -); $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$

Table 3 Bioactive properties of the cocoa powder

	Total phenolic content (mg GAE/100 g)	Total antioxidant capacity (mg TE/100 g)		Total flavonoid content (mg CE/100 g)
		DPPH	CUPRAC	
S1	920.10 ± 5.20^c	1686.19 ± 3.24^c	4949.24 ± 31.50^c	299.32 ± 4.00^c
S2	896.06 ± 7.81^d	1502.62 ± 9.73^d	4823.23 ± 31.50^d	274.01 ± 3.02^d
S3	712.76 ± 6.89^e	1049.33 ± 6.49^e	4014.63 ± 18.19^e	182.38 ± 3.02^e
S4	1898.19 ± 5.20^b	2800.70 ± 9.73^b	7753.08 ± 54.57^b	760.95 ± 1.51^b
SC	2789.15 ± 7.81^a	4353.52 ± 8.58^a	10357.39 ± 36.38^a	1077.72 ± 15.11^a

GAE, gallic acid equivalent; CE, catechin equivalent; TE, Trolox equivalent; Data are means ± standard deviations of triplicate determinations ($n = 3$). a-e: The different lowercases within the same column show that the results are significantly different ($P < 0.05$)

7.90) and darkened the color (reduction of L* value) of the samples S1, S2, and S3. However, the increase in the pH and ΔE value was much lower for S4 when compared to other samples due to the lower concentration (1% w/w) of the alkali agent (NaOH) used (Table 1). Alkalization from 1 to 6% (w/w) concentration using different alkali combinations caused an increase in ΔE value (ranging from around 3.6 to 23.3) which shows the darkening of the cocoa powder samples compared to the control. Similar data were reported previously by Sioriki et al. [9]. The darkness can be visually perceived, as the biggest differences were noted in the *L** and *b** values (Table 2). NaOH + KOH (4% w/w) combination (S1) gave the darkest color score even though the concentration was lower compared to NaOH + K_2CO_3 (5% w/w) (S2) and K_2CO_3 (6% w/w) (S3). Cocoa powder contains anthocyanins and other compounds that react with bases to form different colors (i.e. brown polymers) [7]. Stronger bases or certain combinations of bases can lead to more intense color changes. The NaOH + KOH combination (S1) might enhance these reactions more effectively than NaOH + K_2CO_3 (S2) or K_2CO_3 alone (S3), resulting in the darkest color. Both NaOH and KOH are strong bases, but they can affect the color development in cocoa powder differently. KOH is often more effective in reacting with polyphenols in cocoa, which might result in more intense color changes or a darker color [25]. This could explain why a 4% w/w NaOH + KOH mixture (S1) might produce a darker color compared to higher concentrations of other combinations, in agreement with other authors like Valverde et al. [25]. The a*/b* ratio represents the intensity of the reddish color in cocoa powder. As can be seen in Table 2, the a*/b* value of non-alkalized cocoa powder is around 0.55, while that of alkalized powders ranged from 0.57 to 0.91. This increase implies an increase in the redness of the alkali-treated cocoa powders.

Bioactive properties of cocoa powders

Cocoa beans contain about 10–18% of polyphenols including high amount of flavonoids on a dry weight basis [26, 27]. Polyphenols found in cocoa-containing foods contribute to the brown cocoa color and bitter taste and are also associated with human health benefits [7].

As seen in Table 3, the total polyphenol content of natural cocoa powders (SC) was 2789.15 ± 7.81 mg GAE/100 g sample. On the other hand, total polyphenol content decreased significantly ($P < 0.05$) depending on the type of alkali solution used. The lowest polyphenol reduction rate was observed in S4 with 1% of NaOH solution (approximately 32%), while the highest decrease rate was observed in S3 with 6% of K_2CO_3 solution (approximately 75%). Also, a similar reduction rate (approximately 67–68%) was observed in S2 with 5% NaOH-K_2CO_3 solutions and S1 with 4% NaOH-KOH solutions. Polyphenols are generally unstable under alkaline conditions, and oxidative destruction is expected. This loss could be caused by the polymerization and production of insoluble high molecular weight pigment compounds that result from the oxidation of polyphenolic compounds to quinones. In addition, under high temperatures and alkaline conditions, their reaction with proteins may cause a decrease in phenolics [28, 29]. Many studies showed that the alkalizing process reduces the amount of polyphenols in cocoa depending on the alkali type and concentration [30]. In a similar research conducted by Alasti et al. [7], the lowest total polyphenol content was observed in the sample alkalized with 3% of K_2CO_3 solution, while the highest one in the sample alkalized with 1% of NaOH solution. In another research, the average decrease in polyphenol content was determined as 20.4% [31], while Miller et al. [1]found the average decrease as 60.5%.

On the other hand, similar changes were observed in the total flavonoid contents of the cocoa powders in this study (Table 3). Compared to natural cocoa powder, the decrease in flavonoid content was determined as 72.3%, 29.4%, 82.3%, and 73.7%, in S1, S2, S3, and S4, respectively. Gültekin-Özgüven et al. [28] found that the percent decrease of total flavonoid was 81% in heavily alkalized samples with calcium carbonate. In another study, it was determined as 60%, 78%, and 89% for lightly alkalized (pH 6.5–7.2), medium alkalized (pH 7.2–7.6), and heavily alkalized (pH 7.6 and higher) cocoa powders, respectively [1]. Andres-Lacueva et al. [32] also reported a 60% reduction in total flavonoid content in cocoa powders under different alkalization conditions. The authors ([1] and [32]) state that they obtained alkalized cocoa samples from different companies and the type of alkali chemicals they used for alkalization are not mentioned in their papers.

Considering the antioxidant capacity, cocoa has different in vitro beneficial effects, such as protecting neurons, stimulating vasodilation, improving insulin secretion, and inhibiting cancer cell proliferation [33]. The antioxidant capacity of the samples was determined using two different methods, namely DPPH and CUPRAC, due to the antioxidant activity occurs by different mechanisms, using a single mechanism-dependent method may not reflect the actual antioxidant capacity [34]. As seen in Table 3, the results showed good accordance between the two antioxidant methods as well as with total phenolics and flavonoids results. Compared to natural cocoa powder, the decrease in antioxidant capacity was determined as approximately 52–61%, 53–65%, 61–75%, and 25–35% in S1, S2, S3, and S4, respectively. It is reported that the antioxidant capacity is highly depending on the origin of cocoa, alkalization process parameters, extraction method, and analysis method [35]. Todorovic et al. [30], report the antioxidant capacity of alkalized cocoa powder as 1.6-1.8-fold less than natural cocoa powder. In another research, the alkalization process reduced antioxidant capacity by 42% for DPPH and 58% for ORAC assay [28]. The decrease in the antioxidant activity might be attributed to the degradation or alteration of phenolic compounds during the alkalization and powder production process [12].

As a result, despite the loss of phenolics, flavonoids, and antioxidants, the alkalization process is desired by food manufacturers. However, labeling the cocoa powder type can give the consumer important information about the quality and functionality of cocoa-based foods [30].

Browning index

Color parameters are generally used as an indicator of browning reactions in cocoa powders, and temperature and alkalization are related to these changes [7, 36]. Figure 2 shows the color changes in the cocoa powders as browning index (OD_{460}/OD_{525}) (a) and the relationship between color and total phenolic amount (b). As seen in the figure, natural cocoa powder (SC) has light brown color with a browning index of 1.37. The alkalization process caused significant color changes ($P<0.05$) in the cocoa powders, and the highest browning index was obtained in S3 (very dark brown) alkalized with 6% of K_2CO_3. The increase in the browning index might be due to rapid degradation of the polyphenols into brown pigments [37]. Therefore, it can be stated that there is a negative relationship between the browning index and total phenolic content. Maillard reaction, which caused an increase in brown pigment formation during alkalization, was mainly responsible for the high browning index (OD_{460}/OD_{525}) of the alkalized samples [37]. On the other hand, since the alkali species used in the alkalization process had different ionization strengths, the Maillard reaction took place at different pH values, and K^+ and Na^+ ions caused changes in the anthocyanin structures, so the samples showed different colors [38]. In similar research, the browning index of non-alkalized cocoa powder was found to be 1.158, while the highest browning index (1.787) was observed in the alkalized sample with 1.5% K_2CO_3 and 0.5% NH_4HCO_3 solutions. Also, the browning index value was found as 1.297 in the sample alkalized with 1% NaOH and 1.747 in the sample alkalized with 3% K_2CO_3 [7]. Moreover, Li et al. [38] found that cocoa powder alkalized with a 2% K_2CO_3 solution had a darker color and lower polyphenolic compounds than powder alkalized with a NaOH solution, and also indicated that the OD_{460}/OD_{525} value of excellent grade cocoa should not be less than 1.1 after fermentation and roasting.

FTIR spectrum of cocoa powders

FTIR was employed to examine the differences in the functional groups in the cocoa powder samples treated with different alkalis. Figure 3A exhibits the vibrational FTIR spectra of the cocoa samples. The bands' region of 1600–1680 cm^{-1} was assigned to Amide I and aromatic ring (amides, aldehydes, ketones, carboxylic acids, and flavonoids) vibrations, while 1500–1580 cm^{-1} region could be attributed to the presence of amide II and NH bending. 1420 and 1240 cm^{-1} bands would be associated with stretching vibrations of aromatic C-C, C-N, -S=O, and CH_3 groups. It can be noticed from Fig. 3A that the peak intensities at 1240 cm^{-1}, which is linked to C-O valent deformation in

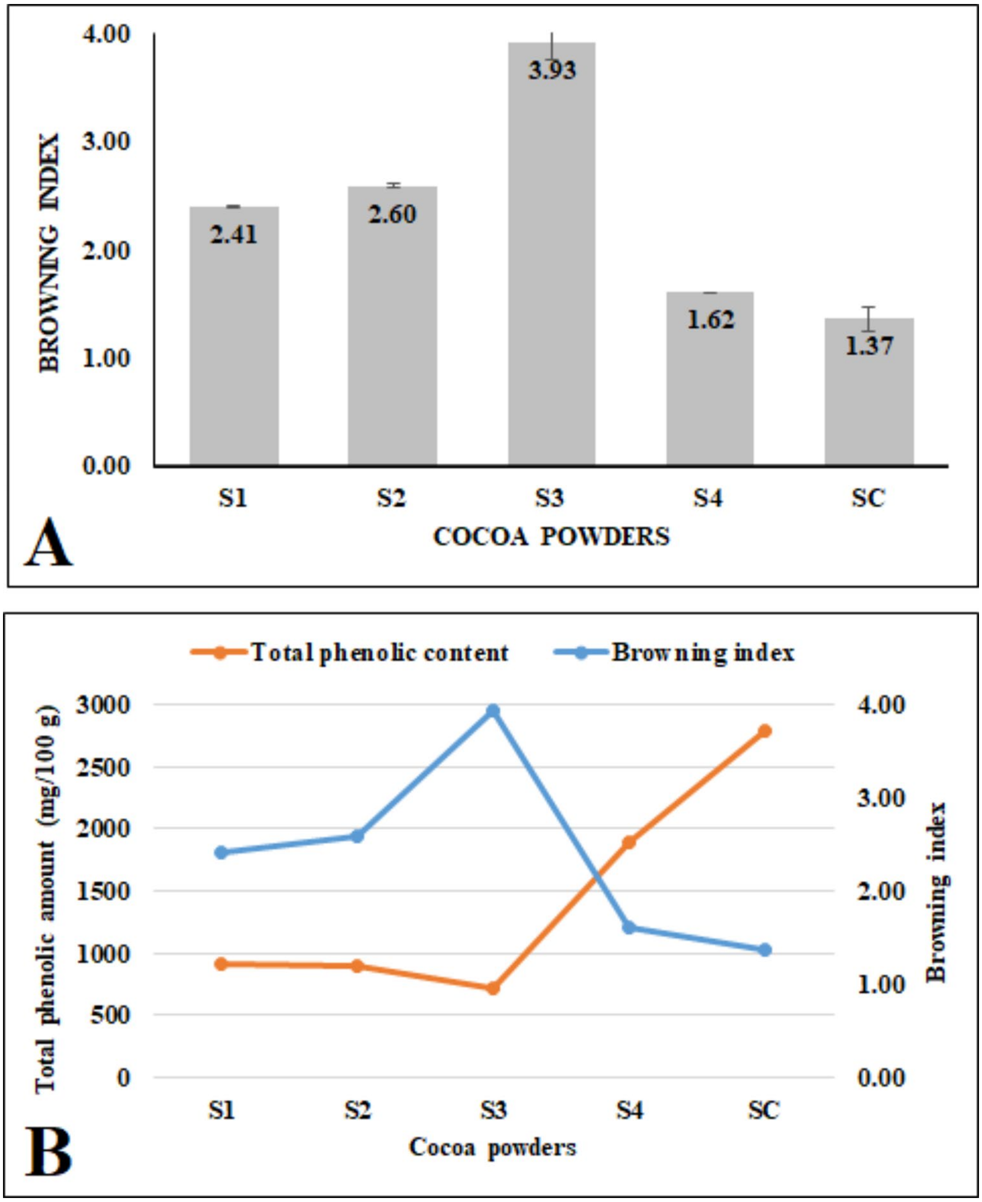


Fig. 2 The browning index of cocoa powders and its relationship with total phenolic content

acetyl groups [39], for S1 and S2 are weaker than other samples. This could be attributed to the effect of different alkalis at different concentrations on these functional groups. The bands around 2915, 2850, and 1750 cm^{-1} might be ascribed to symmetric or asymmetric stretching of CH, CH_2, and C$=$O groups. The absorption band around 3300 cm^{-1} was linked to the stretching of the OH group [15, 22]. The slight differences in the intensities of vibrations of CH_2 methylene groups around 2915 and 2850 cm^{-1} bands revealed that these groups were affected by different alkali agents used. The variations in the intensity of peaks at 2915 cm^{-1} when compared with the SC (untreated sample) might be linked to C-H stretching in CH2 and CH3 groups, mainly in lipids [39]. Similarly, the peak intensity of C$=$O stretching of the carbonyl group of carboxylic acids occurred at around 1750 cm^{-1} and varied slightly. The absorption peaks in the region of 550–600 cm^{-1} corresponded to bending outside the plane of the C–H bond [22, 40].

PCA score plots of the first two principal components of FTIR spectra of cocoa powders treated with different alkalis are shown in Fig. 3B. Two principal components (accounting for 96% of total variance) corresponding to the alkali type were considered in PCA. F1 represents 86% of the total variance, and F2 represents 10% of the total variance. PCA score plots depicted a clear separation among the cocoa samples treated with different alkalis in correlation with FTIR data. The categorization of cocoa powder samples treated by various alkali agents may have been much aided by these spectra observations.

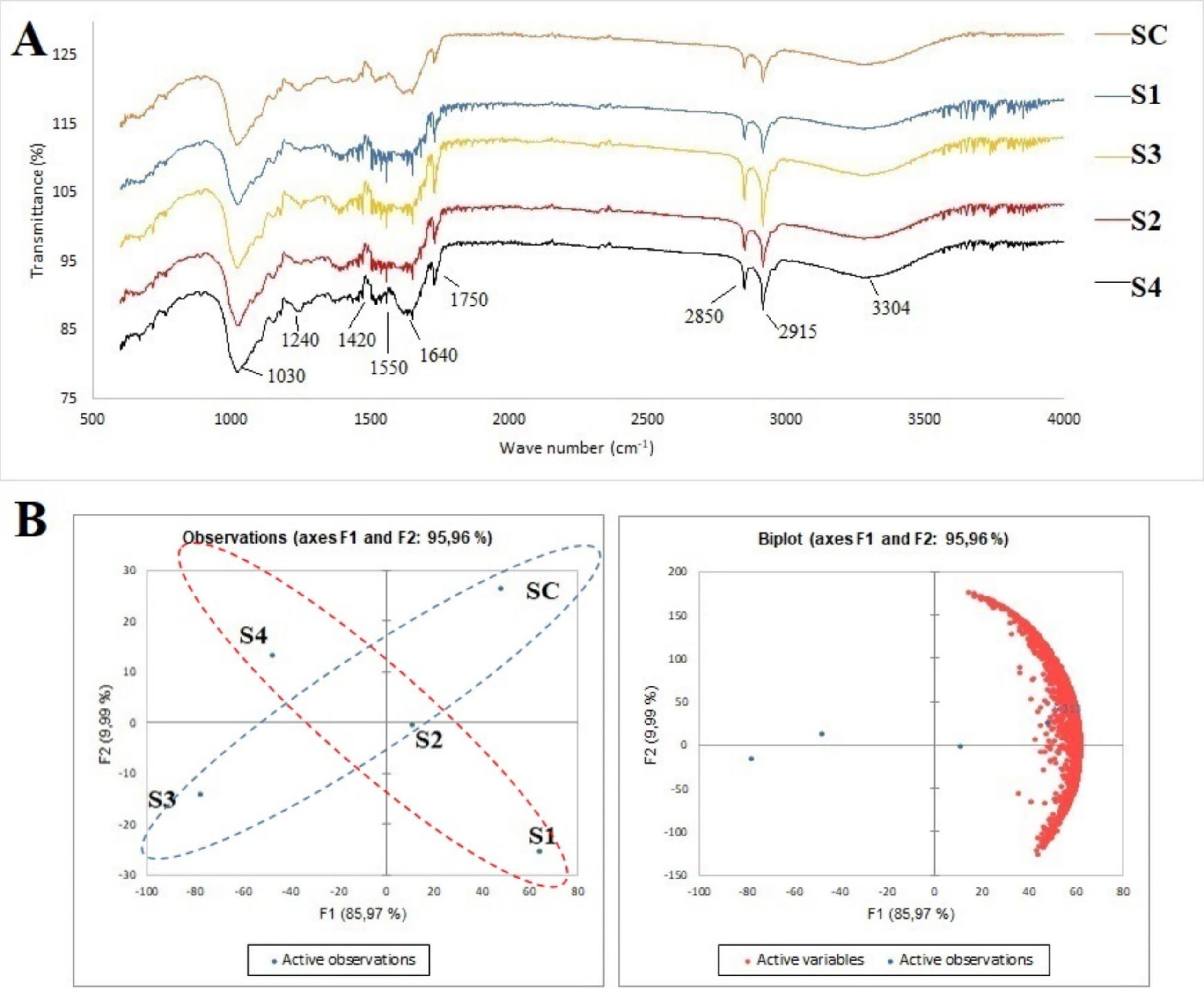


Fig. 3 FTIR spectrums (A) and PCA score plots (B) of the cocoa powders

Thermal behavior of cocoa powders

Thermal characterization is an essential factor in adding cocoa powder to various food formulations The powder samples were kept at room temperature prior to the DSC analysis to start with their amorphous state. A prominent endothermic transition peak was detected from all DSC curves (Fig. 4) with varied onset (T_{onset}) (ranging from around 30 to 34 °C) and peak (T_{peak}) temperatures (varied from 36 to 45 °C). The phase transitions at different temperatures are presented in Table 4. Notable endothermic transition peaks were assigned as melting curves with an onset temperature varied from around 30 to 34 °C (Fig. 4). Calva-Estrada et al. [41] reported similar findings with slight differences. They indicated the onset and peak temperatures of dark chocolate samples around 30 and 33 °C, respectively. The slight differences between the findings of this study to those of their results might be attributed to the differences in chemical structure (i.e., fat content) of the dark chocolate and alkalized cocoa powder samples. The decomposition temperature was noted as around 188 °C for all samples. It can be observed from Fig. 4 that the type of alkali and their concentration affected the intensity and position of the melting curve, with a clear trend of decreasing melting points in the order of S4, S3, S2, and S1.

Morphological properties of cocoa powders

The particle size is an important parameter affecting functional, physical, and powder properties. It was observed that the d_{90} value of cocoa powder samples ranged from 54 to 75 µm (Table 2).

Figure 5 shows the scanning electron micrographs of cocoa powder samples treated with different alkali solutions.

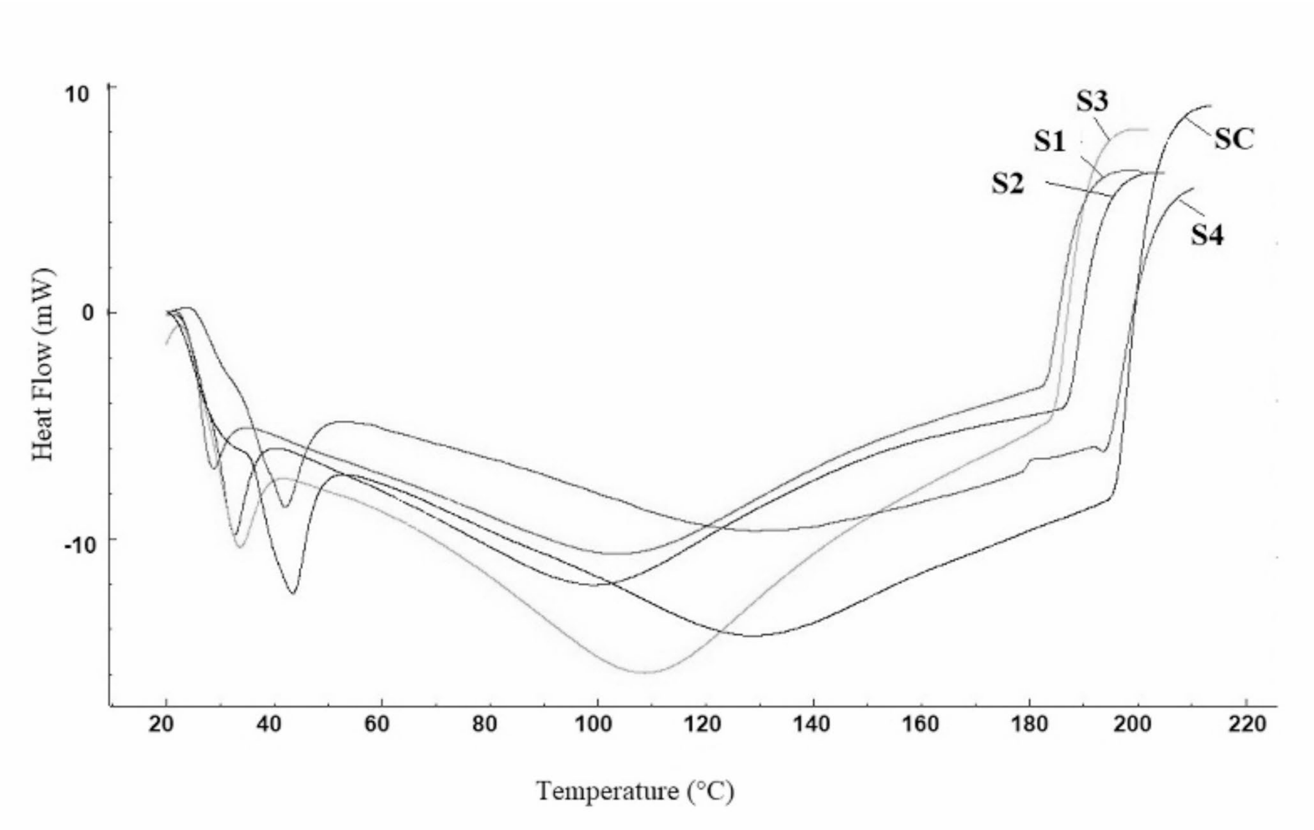


Fig. 4 DSC thermogram of the cocoa powders

Table 4 Thermal properties of the cocoa powders

Sample	T_{onset} (°C)	T_{peak} (°C)	$T_{melting}$ (°C)
S1	30.1	36.4	104.5
S2	29.7	37.7	97.0
S3	33.5	41.8	108.7
S4	34.0	44.7	121.6
SC	32.0	42.0	117.5

It was observed from the images that the surface microstructure of cocoa particles was not affected by different alkali treatments significantly. Thus, it could be noted that using different alkalis did not have any detrimental effects on the microstructure of cocoa particles. The particles had irregular and oblong shapes. The surface of the particles was seen as irregular with little porosity. It can be seen from the images that some of the particles grouped to form agglomerates revealing that the particles have sticky nature. This behavior agrees with the findings of Germann, Stark and Hofmann [42]. They reported that the alkalization led to agglomeration with brown high-molecular-weight compounds.

Powder flow properties

Powder flow is an important attribute of powder systems from the industrial point of view and needs to be evaluated to design the processes and process equipment. The PFT gives the flow function curve which is plotted between the major consolidation stress applied and the unconfined yield strength of the powder sample. The flow index (FI) is the reciprocal of the slope of the flow function curve. When FI falls between 2 and 4, the powder is classified as cohesive, and when it is between 4 and 10, it is classified as easy flow [43]. Based on the results given in Fig. 6A, one can state that the alkalization process adversely affected the flowability of cocoa powders compared with the control sample, even though all the samples fall into cohesive region. Similarly, Ali et al. [10] and Lapčík et al. [44] report that the cocoa powders are very cohesive and have poor flow properties. The degree of cohesiveness as depicted from Fig. 6A can be classified as SC (control) < S2 (%2,5 + 2,5NaOH + K2CO3) < S4 (%1NaOH) < S1 (%2 + 2NaOH + KOH) < S3 (%6K2CO3) from less cohesive to more cohesive. Several factors, such as particle size, shape, and chemical composition of the powder samples, cause variations in flow behavior [45]. In this study, since the particle size and shape properties of the samples do not differ much (Fig. 5), the primary reason to the variations in the flow behavior might be attributed to the alterations in the chemical compositions of the particles. The use of different alkali agents may explain the change in cohesiveness due to the changes in chemical composition on

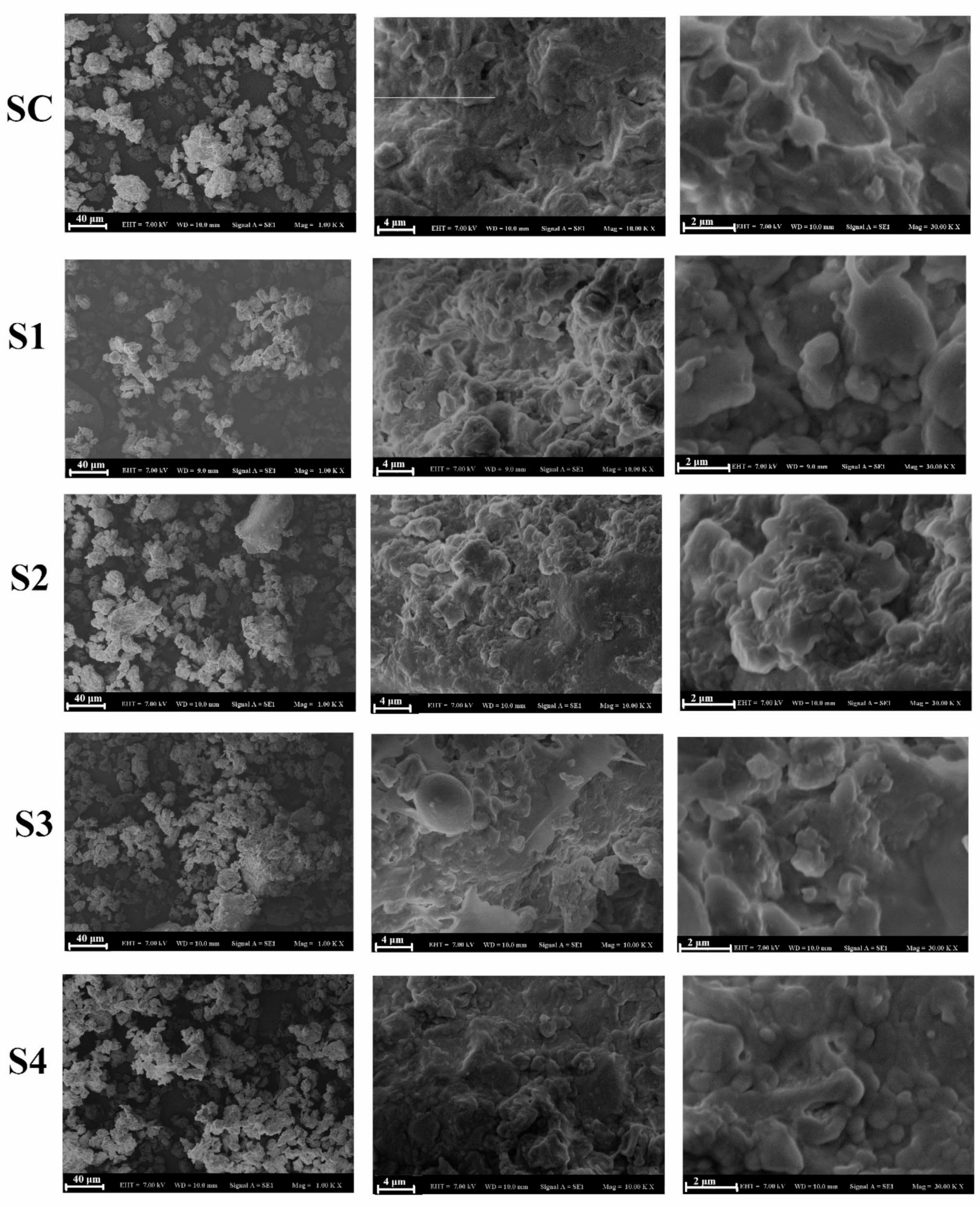
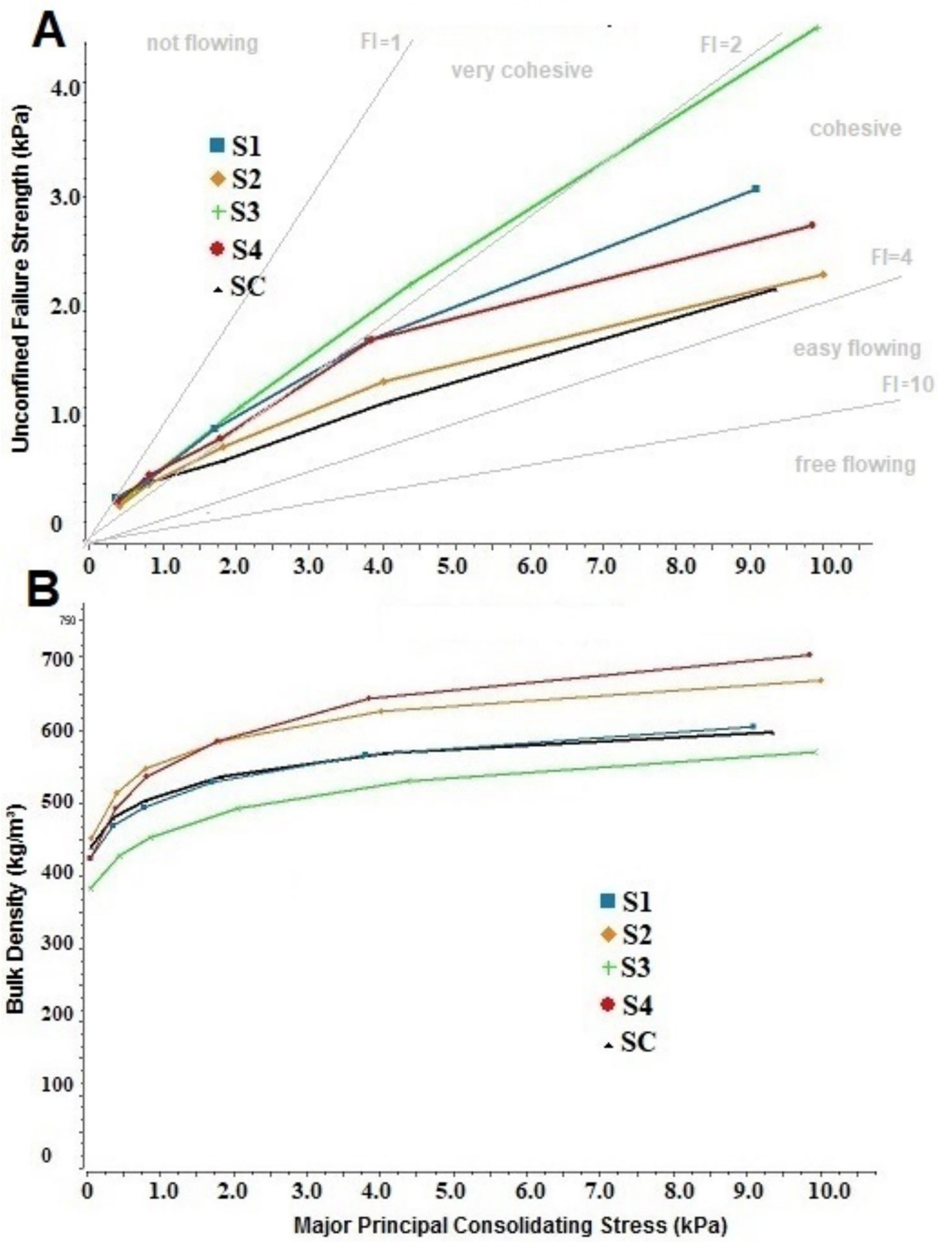


Fig. 5 SEM images of the cocoa powders

Fig. 6 Flow properties of the cocoa powders. A: Flow properties, B: Bulk density curves



the surface of the particles. An increased interparticle cohesiveness due to the intermolecular forces of attraction might be the reason for decreased flowability. Ribeiro, Costa and Afonso [46]observed that changing the chemical structure by adding maltodextrin to cocoa pulp powder improved the flowability.

Figure 6B exhibits a plot between the bulk density and normal stress for cocoa powder samples. A sharp increase in bulk density values was noted with a slight increase in normal stress initially. However, the bulk density graph flattens as the normal stress increases further. The degree of the increase in the density of a powder as a function of the applied stresses might be attributed to its degree of compaction [47]. In this study alkalization process affected the bulk density values. The reason might be altering the molecular weight of particles by adding alkali agents and alkalization process [47].

The findings of this study show that the alkalization process affected the physicochemical, functional, microstructural, and flow properties of cocoa powder. It was found that alkaline type and concentration affected the pH. A negative correlation between pH value and a*/b* value was noticed. Variations in the color characteristics are obtained by using different alkali agents and their concentrations due to the oxidation reactions involving polyphenols, proteins, some saccharides, and other components, which may vary based on the pH and the type of alkali used. A slight variation in particle size was noticed, while no visible change in the microstructure of particles was noticed from SEM images. The flow and bulk density properties of the alkalized cocoa powder samples varied depending on the type of alkalis and their concentrations. As a result, all alkalized samples showed cohesive behavior with increasing cohesiveness depending on the alkalization conditions when compared with the control sample.

Conclusion

The current study reported the alkalization process's effect using different alkali agents on cocoa powders' physicochemical, functional, microstructural, and flow properties. It was found that the alkalization process and the type of alkali agent used did not have much detrimental impact on the microstructure of cocoa particles. However, it was found that the type of alkali agents and alkalization process affect color, bioactive, chemical, thermal, and flow properties. While the flow behavior of cocoa powder samples was observed in the cohesive region, alkali treatment increased the stickiness and adversely affected the flowability of powder samples. Cohesive structure and low flow ability can be problem in flowing of cocoa powder into pipes or homogenization of powders by mixing in the mixer. Therefore, during process optimizations alkalization level should be also considered for eliminating of problems resulted from flowability of cocoa powder.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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