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Impact of alkalization conditions on the phytochemical content of cocoa powder and the aroma of cocoa drinks

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ABSTRACT

Alkalization is an important process in cocoa powder production that affects color and flavor. In this study, the impact of alkalization temperature (60, 70, 80, 90, 100 °C), NaOH concentration (0.59, 1.17, 2.34, 3.59% w/w of cocoa powder) and alkalization time (1 and 10 min) on the physicochemical properties (pH, color) and phytochemical profile (theobromine, caffeine, epicatechin, catechin) of cocoa powder were investigated, while the aroma was studied on the corresponding cocoa drinks. High-performance liquid chromatography coupled to an ultra-violet detector (HPLC-UV) was used for screening the non-volatiles and headspace solid - phase microextraction - gas chromatography - mass spectrometry (HS-SPME-GC-MS) for the aromatic compounds. Major changes of the cocoa properties occurred during the first minute of alkalization. Increase of temperature and alkali concentration generally reduced the levels of epicatechin and the lightness (L*), while the pH of the cocoa powder was affected by changing the alkali concentration. On the other hand, the reddish (a*) and yellowish (b*) color component values and theobromine levels were not significantly affected by varying temperature and alkali concentration. A higher temperature did not affect the concentration of the volatile compounds, while a decrease in certain chemical classes was observed by increasing the alkali concentration.

1. Introduction

Alkalization is a processing technique for cocoa that has been applied for almost two centuries. During this process cocoa is treated with a food-grade alkali solution increasing the pH, producing darker colors and more intense flavors. Therefore, this process is used to reduce the acidity, astringency and bitterness of cocoa, thus improving its palatability. Moreover, alkalization improves the dispersibility of cocoa powder, while it changes the color, ranging from brown, red/brown to brown/black, depending on the reaction conditions. These changes are mainly caused by degradation of bioactive compounds, such as polyphenols (i.e. flavan-3-ols, anthocyanins and proanthocyanidins), and methylxanthines (i.e. theobromine, caffeine and theophylline) (Li et al., 2012; Miller et al., 2008).

Several studies showed that increasing alkali concentration, alkalization temperature and time, resulted in higher decreases of total polyphenols and methylxanthines (Li et al, 2012, 2014; Stanley et al., 2015). However, factors such as alkali agent, matrix (cocoa nibs, liquor, cake), roasting conditions, cocoa origin and phytochemical content in raw material can affect the physicochemical properties and therefore, the flavor of the final product. Alkalization is carried out at temperatures ranging from 75 to 125 °C depending on desired color, using sodium, potassium, ammonium hydroxide, carbonate and bicarbonate or magnesium carbonate and oxide in an amount of 1–3% of the weight of the cocoa (Moser, 2015). The process is usually performed on cocoa nibs before roasting for broader ranges of color and flavor. However, this method has a disadvantage, since the cocoa butter entrapped in the cocoa nibs might react with the alkali (saponification) altering its final color, flavor and crystallization characteristics (De Ginestel, 1998). Therefore, deodorization of cocoa butter is required which causes yield loss and increased cost. Though less popular, cocoa cake alkalization after cocoa butter separation is a cost-effective process but also has

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Abbreviations: HPLC, High-Performance Liquid Chromatography; UV, Ultra-Violet detection; HS, Headspace; SPME, Solid - Phase Microextraction; GC, Gas Chromatography; MS, Mass Spectrometry.

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drawbacks, as flavors might be less pronounced or vanish as previously roasted cocoa is reprocessed (Moser, 2015).

The purpose of this research was two-fold and concerned initially the investigation of alkalization conditions, alkalization temperature – time and alkali concentration – time, on the physicochemical properties and phytochemical profile of cocoa powder. The potential impact, studied for first time, on the flavor related compounds of the corresponding cocoa drinks. Commercial roasted cocoa powder was alkalized on lab-scale. Analyses such as pH, color and HPLC-UV were performed on both non-alkalized and alkalized cocoa powders, while the volatile aroma compounds (HS-SPME-GC-MS) were detected and semi-quantified in the corresponding cocoa drinks.

2. Materials and methods

2.1. Materials and reagents

Commercial fat-reduced cocoa powder (10–12% w/w) was supplied by Cargill (Schiphol, Netherlands). Analytical grade NaOH (98%) for alkalization was purchased from Chem-Lab NV (Zedelgem, Belgium) and demineralized water was purified using Elix type 2 pure water system 20 from Merck Millipore (Overijse, Belgium). Methanol (≥99.8%) and formic acid (98%) were obtained from Fischer Scientific (Loughborough, UK), while *n*-hexane (≥97%), acetone (99.8%) and acetic acid (99.8%) were purchased from Acros Organics (Geel, Belgium). Reference compounds of theobromine, caffeine, epicatechin and catechin (>98% purity) were provided by Sigma Aldrich (Overijse, Belgium). All the other chemicals were of highest grade commercially available.

2.1.1. Cocoa powder alkalization

Cocoa powder was dispersed in a round bottom flask with demineralized water (1:9 w/v). While stirring, the mixture was heated to the desired alkalization temperature and a predetermined volume of 2.20 mL NaOH solution 4M was added to bring the starting pH to 8, according to Stanley et al. (2015). This volume of 2.20 mL corresponded to a concentration of NaOH 2.34% w/w of cocoa powder, for the specific cocoa powder used. The experiments were divided into two parts, in each of which the conditions of alkali concentration or temperature were constant, according to the conditions used in the protocol of Stanley et al. (2015), while changing alternately the other condition. In the first part of the experiment, the alkalization was conducted under a constant alkali concentration of NaOH 2.34% w/w, while varying the temperature (60, 70, 80, 90 and 100 °C). In the second part, the alkalization was conducted under a constant temperature of 90 °C, while varying the volume of NaOH solution (0.55, 1.10, 2.20, 3.30 mL corresponded to 0.59, 1.17, 2.34, and 3.59% w/w). In both experiments, the process lasted for a fixed time (1 and 10 min). After the alkalization reaction, the solution was cooled over ice. The alkalization tests were performed in triplicate and the samples obtained were freeze-dried (VaCo 5 ZIRBUS, Bad Grund, Germany) at approximately 0.04 mbar for a week. The resulting cocoa powders were stored at −24 °C before analyses. All analyses were performed once for each independent repetition, except for color which was performed in triplicate, for each independent repetition.

2.1.2. pH and color

The pH of each sample was measured by dispersing cocoa powder (1.0 g) in hot demineralized water (9 mL, 80 °C). The mixture was cooled down to room temperature (19–21 °C) and measured using as reference a 3-point calibration against standardized buffers (pH 4, 7 and 10) in a HI-2020 edge pH meter (Hanna Instrument, Woonsocket, RI). The color of the samples was analyzed using a Minolta CM-2500d (Konica Minolta Sensing Osaka, Japan) spectrophotometer with an observation angle of 10° and a D65 light source. Plastic petri dishes (8 cm diameter) were filled with cocoa powder and were tightly closed to prevent air gaps. The measurement was carried out at three different spots, and lightness L* (ranging from black = 0 to white = 100), a* (ranging from green = −120 to red = +120) and b* (ranging from blue = −120 to yellow = +120) color components were obtained. Color difference with the non-alkalized cocoa powder (ΔE) as the control, was calculated as follows: $\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$ (Deeth & Lewis, 2017).

2.2. Quantitative analysis of phytochemicals

2.2.1. Fat removal and extraction of phytochemicals from cocoa powder

Prior to the extraction, cocoa powder was defatted according to the method of Tuenter et al. (2020), with slight modifications. Five grams of cocoa powder were suspended in 25 mL of *n*-hexane. The mixture was vortexed and sonicated for 10 min (at room temperature and frequency at 37 kHz), followed by centrifugation at 4500 rpm for 5 min (at 4 °C). The defatting procedure was repeated three times and the defatted cocoa powder was dried at room temperature for at least 48 h, protected from light. Extraction of 0.5 g of defatted cocoa powder was carried out with 10 mL of a mixture of acetone: water: acetic acid (70.0:29.8:0.2). The sample was vortexed and sonicated for 30 min. Next, the samples were centrifuged for 5 min at 4 °C at a speed of 4500 rpm and the supernatant was collected. This extraction procedure was repeated three consecutive times and the obtained extracts were combined and additionally centrifuged for another 5 min to remove any remaining cocoa solids.

2.2.2. HPLC-UV analysis

To quantify the levels of theobromine, caffeine, epicatechin and catechin in the cocoa powders, a HPLC system of the Agilent 1200 series was used. The system was equipped with an autosampler, quaternary pump, and DAD (diode array detector) (Agilent Technologies Belgium NV, Diegem, Belgium). Separation was obtained with an XBridge C18 column (250 × 4.6 mm, 5 μm) (Waters, Milford, Ma, USA), using water + 0.1% formic acid (A) and methanol + 0.1% formic acid (B) as mobile phase. The following gradient was applied: 0 min 10% B, 14.5 min 45% B, 15.5–17.5 min 100% B, 19–25 min 10% B.

The mobile phase flow rate was 1 mL/min, - the column was kept at a temperature of 40 °C and the injection volume was 20 μL. UV detection was carried out at 210 nm for epicatechin (Tr 17.7 min) and catechin (Tr 12.0 min) and at 280 nm for caffeine (Tr 15.0 min) and theobromine (Tr 7.6 min). A calibration curve of each component was created using the reference compounds, in the range of 0.28–70 μg/mL for theobromine ($y = 56.095x - 10.344$, $R^2 = 0.999$ and $y = 61.079x + 1.2208$, R2 = 0.9999), in the range of 0.21–51 μg/mL for caffeine ($y = 64.905x - 12.335$, $R^2 = 1.000$ and $y = 52.934x – 5.0984$, R2 = 1.0000), in the range of 0.7–56 μg/mL for epicatechin ($y = 171.3x + 91.882$, $R^2 = 0.9995$ and $y = 155.36x + 142.44$, R2 = 0.9984) and catechin ($y = 186.97x + 47.67$, $R^2 = 1.000$ and $y = 371.87x + 94.831$, R2 = 0.9994), with x referring to the concentration of the analyzed sample in μg/mL and y the peak area. Based on these equations the respective phytochemicals were quantified in the defatted cocoa samples and the effect of temperature, or the effect of the alkali concentration applied during alkalization, respectively, were determined.

2.3. Semi-quantitative analysis of volatile compounds

2.3.1. HS-SPME-GC-MS

For the preparation of the cocoa drinks 2 g of both alkalized and non-alkalized cocoa powder (of 1 min alkalization) was dispersed in 18 mL of water and stirred on a stirring plate at room temperature for 5 min on a moderate speed until homogenization. While stirring, 2 mL of the suspension was transferred to a GC-MS vial. The cocoa drinks corresponded to a suspension of 0.22 g cocoa powder in 2 mL of water. The aroma profiles were analyzed with a HS-SPME-GC-MS system, applying the method of Lemarcq et al. (2020). 4-Methylpyridine (1.045 μL of a 0.957

Table 1
Color (CIELAB) and pH of non-alkalized and alkalized cocoa powders after 1 and 10 min alkalization at constant alkali concentration of NaOH 2.34% w/w of cocoa powder and different alkalization temperatures.

Treatment	Color (SCE)					pH
	L*	a*	b*	a*/b*	ΔE	
Non-alkalized	$42.52 \pm 0.75^{a,A}$	$11.65 \pm 0.33^{a,A}$	$32.80 \pm 1.29^{a,b,B}$	$0.36 \pm 0.01^{a,b,A}$	0.00	$5.93 \pm 0.01^{a,A}$
60 °C						
1 min	28.45 ± 0.60^{b}	11.03 ± 0.36^{a}	38.41 ± 3.06^{c}	0.29 ± 0.02^{d}	15.19 ± 1.37	8.66 ± 0.03^{d}
10 min	24.32 ± 0.88^{B}	$13.75 \pm 0.66^{B,C}$	38.38 ± 0.66^{A}	0.36 ± 0.01^{A}	19.29 ± 0.93	8.61 ± 0.02^{E}
70 °C						
1 min	22.39 ± 0.94^{c}	11.71 ± 0.95^{a}	34.59 ± 1.23^{b}	0.34 ± 0.02^{a}	20.23 ± 1.44	8.60 ± 0.07^{d}
10 min	18.97 ± 0.87^{C}	15.01 ± 0.78^{C}	$31.81 \pm 1.39^{B,C}$	0.47 ± 0.02^{C}	23.85 ± 1.15	8.65 ± 0.02^{E}
80 °C						
1 min	$21.24 \pm 1.82^{c,d}$	13.00 ± 0.64^{b}	34.30 ± 1.86^{b}	$0.38 \pm 0.04^{b,c}$	21.46 ± 1.10	$8.58 \pm 0.05^{c,d}$
10 min	19.36 ± 1.67^{C}	13.67 ± 0.94^{B}	$31.74 \pm 1.80^{B,C}$	$0.43 \pm 0.05^{B,C}$	23.34 ± 1.24	8.50 ± 0.03^{D}
90 °C						
1 min	21.27 ± 1.09^{c}	9.63 ± 0.39^{c}	28.71 ± 1.04^{d}	0.33 ± 0.01^{a}	21.74 ± 1.50	$8.48 \pm 0.02^{b,c}$
10 min	19.52 ± 0.67^{C}	12.01 ± 1.21^{A}	30.50 ± 1.64^{C}	$0.39 \pm 0.03^{A,B}$	23.14 ± 0.38	8.34 ± 0.02^{C}
100 °C						
1 min	19.69 ± 1.03^{d}	12.77 ± 0.62^{b}	31.54 ± 1.01^{a}	0.41 ± 0.03^{c}	22.89 ± 1.18	8.39 ± 0.04^{b}
10 min	15.89 ± 1.07^{D}	$14.83 \pm 1.21^{B,C}$	26.69 ± 1.41^{D}	0.56 ± 0.08^{D}	27.56 ± 2.47	8.22 ± 0.02^{B}

Note: All results represent the mean ± SD ($\bar{X}$ ±SD) of independent measurements ($n = 3$).
Color was performed in triplicate, for each independent repetition.
Small lowercase letters (a–d), within one column, indicate significant differences ($p < 0.05$) for 1 min of alkalization, while capital lowercase letters (A-D), within one column, indicate significant differences ($p < 0.05$) for 10 min of alkalization between alkalization temperatures following one-way ANOVA and post hoc test.

Table 2
Color (CIELAB) and pH of non-alkalized and alkalized cocoa powders after 1 and 10 min alkalization at constant temperature of 90 °C and different alkali concentrations.

Treatment	Color (SCE)					pH
	L*	a*	b*	a*/b*	ΔE	
Non-alkalized	$42.52 \pm 0.75^{a,A}$	$11.65 \pm 0.33^{a,A,C}$	$32.80 \pm 1.29^{a,c,B}$	$0.36 \pm 0.01^{a,A}$	0.00	$5.93 \pm 0.01^{a,B}$
0.59% w/w NaOH						
1 min	36.06 ± 0.63^{b}	$10.69 \pm 0.24^{a,b}$	35.01 ± 0.66^{a}	0.31 ± 0.01^{a}	6.94 ± 0.92	$6.47 \pm 0.05^{b,B}$
10 min	32.87 ± 0.29^{B}	10.34 ± 0.20^{B}	35.16 ± 0.91^{A}	0.29 ± 0.01^{A}	10.17 ± 0.97	6.67 ± 0.04^{B}
1.17% w/w NaOH						
1 min	35.07 ± 1.07^{b}	10.16 ± 0.34^{b}	27.71 ± 1.30^{b}	0.37 ± 0.01^{a}	9.35 ± 1.22	7.13 ± 0.02^{c}
10 min	32.81 ± 1.68^{B}	10.36 ± 0.30^{B}	28.02 ± 2.04^{B}	0.37 ± 0.02^{B}	11.35 ± 0.57	6.98 ± 0.03^{C}
2.34% w/w NaOH						
1 min	22.41 ± 1.01^{c}	10.35 ± 0.99^{b}	32.10 ± 1.31^{c}	0.32 ± 0.02^{a}	20.22 ± 0.36	8.46 ± 0.05^{d}
10 min	18.93 ± 2.10^{C}	12.45 ± 1.11^{C}	29.75 ± 1.33^{B}	0.42 ± 0.07^{B}	23.94 ± 2.10	8.23 ± 0.07^{D}
3.59% w/w NaOH						
1 min	10.57 ± 1.14^{c}	9.86 ± 0.39^{b}	17.87 ± 0.66^{d}	0.56 ± 0.06^{b}	35.33 ± 1.99	9.20 ± 0.04^{e}
10 min	7.60 ± 0.73^{D}	11.12 ± 0.65^{C}	12.88 ± 0.91^{C}	0.87 ± 0.08^{C}	40.24 ± 1.72	8.89 ± 0.06^{E}

Note: All results represent the mean ± SD ($\bar{X}$ ±SD) of independent measurements ($n = 3$).
Color was performed in triplicate, for each independent repetition.
Small lowercase letters (a–e), within one column, indicate significant differences ($p < 0.05$) for 1 min of alkalization, while capital lowercase letters (A-E), within one column, indicate significant differences ($p < 0.05$) for 10 min of alkalization between alkali concentrations following one-way ANOVA and post hoc test.

mg/mL solution) was added as internal standard in the vials with the suspensions of cocoa powders and they were incubated in a thermostatic incubator at 60 °C for 10 min at 250 rpm. The divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Sigma-Aldrich N.V., Bornem, Belgium) was conditioned at 270 °C for 30 min before the analysis. The volatiles present in the headspace were extracted through exposure to the SPME fiber at 60 °C for 25 min. Split mode was applied (ratio 5:1) and helium (1 ml/min) was used as carrier gas. Desorption took place in the inlet of the GC system at 270 °C for 5 min. The extracted volatiles were subjected to a set time/temperature profile (5 min at 35 °C; 35–182 °C–3 °C/min; 182–240 °C–6 °C/min; 7 min at 240 °C) on a polar DB-WAX UI column (60 m × 250 μm, 0.25 μm) (Agilent Technologies Belgium NV, Diegem, Belgium). The total ion current (70 eV) was measured, without solvent delay, over an m/z range of 40–250. The analysis was performed in triplicate for each independent repetition.

Two different approaches were applied to identify the volatiles. Both a comparison of experimental (KI_{exp}) and from literature (KI_{lit}) derived Kovats indices, and mass spectra experimentally determined and reported in the NIST 14 library (Agilent Technologies Belgium NV) were performed. Afterwards, semi-quantitative concentrations of aromatic cocoa volatiles were calculated and peaks above the limit of quantification (signal-to-noise ratio above 10) were reported as μg equivalents of 4-methylpyridine/g of cocoa drink.

2.4. Statistical analysis

To detect significant differences ($p < 0.05$) between the different alkalization treatments, data of both HPLC-UV and HS-SPME-GC-MS were analyzed using one-way ANOVA by SPSS software (SPSS Inc., Chicago, USA). Equality of variances was checked by means of a Levene test. Tukey's or Dunnett's T3 post-hoc tests were carried out, in case the assumption was met or not, respectively. The test was performed with 95% confidence interval and each result is reported as mean ± standard deviation.

3. Results and discussion

3.1. pH and color

3.1.1. Effect of alkalization temperature

As expected, alkalization greatly darkened the color of cocoa powder and rapidly increased its pH (Table 1). All alkalized cocoa powders reached a pH above 8, meaning that they were in the range of heavily alkalized powders (Miller et al., 2008). Alkalization under constant alkali concentration of NaOH 2.34% w/w, altered the color since all alkalized powders had a ΔE value substantially above 1 when compared to the non-alkalized powder, which can be visually perceived darker from consumers, as the biggest differences were observed in the $L*$ value (Stanley et al., 2015).

Indeed, in the very first minute of the reaction there was a major drop (33–54%) of the $L*$ value of alkalized cocoa powders, followed by a smaller decrease (<20%) after 10 min for all applied temperatures.

The alkaline conditions and high temperatures during alkalization play an important role in darkening of cocoa powder. Under such conditions, the oxidation of polyphenols into quinones is accelerated resulting in polymerization or formation of high molecular weight dark compounds (Li et al., 2014). Furthermore, under these conditions, non-enzymatic Maillard reactions might also take place, which yield brown pigments and produce various α-dicarbonyl compounds that contribute to the development of other side reactions such as the

Table 3

Levels of theobromine, caffeine, epicatechin and catechin (mg/g defatted cocoa powder) of non-alkalized and alkalized cocoa powders after 1 and 10 min of alkalization at constant alkali concentration of NaOH 2.34% w/w of cocoa powder and different alkalization temperatures.

Treatment	Theobromine	Caffeine	Epicatechin	Catechin
Non-alkalized	$19.67 \pm 4.14^{a,A}$	$2.77 \pm 0.24^{a,A}$	$1.41 \pm 0.02^{a,A}$	$0.26 \pm 0.01^{a,A}$
60 °C				
1 min	22.59 ± 1.49^{a}	2.15 ± 0.14^{b}	0.93 ± 0.08^{b}	$0.37 \pm 0.09^{a,b}$
10 min	22.48 ± 1.07^{A}	2.13 ± 0.07^{B}	0.56 ± 0.05^{B}	0.33 ± 0.05^{A}
70 °C				
1 min	22.14 ± 0.87^{a}	2.09 ± 0.10^{b}	0.43 ± 0.10^{c}	0.52 ± 0.06^{b}
10 min	21.64 ± 1.79^{A}	2.16 ± 0.08^{B}	0.36 ± 0.03^{C}	0.59 ± 0.07^{B}
80 °C				
1 min	19.95 ± 2.32^{a}	2.05 ± 0.32^{b}	0.45 ± 0.09^{c}	0.48 ± 0.04^{b}
10 min	20.55 ± 0.65^{A}	2.03 ± 0.02^{B}	0.29 ± 0.02^{C}	$0.64 \pm 0.11^{B,C}$
90 °C				
1 min	19.90 ± 1.03^{a}	1.96 ± 0.10^{b}	0.28 ± 0.08^{c}	0.73 ± 0.05^{c}
10 min	23.11 ± 3.27^{A}	2.23 ± 0.30^{B}	0.29 ± 0.02^{C}	0.95 ± 0.09^{D}
100 °C				
1 min	18.29 ± 1.27^{a}	1.79 ± 0.12^{b}	0.27 ± 0.05^{c}	0.72 ± 0.07^{c}
10 min	20.49 ± 1.05^{A}	1.98 ± 0.11^{B}	0.30 ± 0.02^{C}	$0.84 \pm 0.06^{C,D}$

Note: All results represent the mean ± SD ($\bar{X}$ ±SD mg/g) of independent measurements (n = 3).

Small lowercase letters (a–c), within one column, indicate significant differences ($p < 0.05$) for 1 min of alkalization, while capital lowercase letters (A-D), within one column, indicate significant differences ($p < 0.05$) for 10 min of alkalization between alkalization temperatures following one-way ANOVA and post hoc test.

Table 4

Levels of theobromine, caffeine, epicatechin and catechin (mg/g defatted cocoa powder) of non-alkalized and alkalized cocoa powders after 1 and 10 min of alkalization at constant temperature of 90 °C and different alkali concentrations.

Treatment	Theobromine	Caffeine	Epicatechin	Catechin
Non-alkalized	$19.67 \pm 4.14^{a,A}$	$2.77 \pm 0.24^{a,A}$	$1.41 \pm 0.02^{a,A}$	$0.26 \pm 0.01^{a,A}$
0.59% w/w NaOH				
1 min	21.69 ± 0.66^{a}	2.71 ± 0.18^{a}	1.25 ± 0.07^{a}	0.25 ± 0.09^{a}
10 min	21.15 ± 0.42^{A}	2.81 ± 0.05^{A}	$1.20 \pm 0.02^{A,B}$	0.40 ± 0.07^{A}
1.17% w/w NaOH				
1 min	19.29 ± 0.93^{a}	2.61 ± 0.25^{a}	1.06 ± 0.09^{a}	$0.44 \pm 0.08^{a,c}$
10 min	20.66 ± 0.98^{A}	2.81 ± 0.59^{A}	1.04 ± 0.25^{B}	0.72 ± 0.11^{B}
2.34% w/w NaOH				
1 min	18.41 ± 1.95^{a}	2.04 ± 0.24^{b}	0.26 ± 0.10^{b}	0.71 ± 0.12^{b}
10 min	15.95 ± 0.53^{A}	1.73 ± 0.17^{B}	0.19 ± 0.04^{C}	0.66 ± 0.09^{B}
3.59% w/w NaOH				
1 min	18.48 ± 0.87^{a}	2.28 ± 0.03^{a}	0.22 ± 0.05^{b}	0.57 ± 0.03^{c}
10 min	16.52 ± 0.65^{A}	1.89 ± 0.14^{B}	n. d.	0.30 ± 0.05^{A}

Note: All results represent the mean ± SD ($\bar{X}$ ±SD mg/g) of independent measurements (n = 3).

Small lowercase letters (a–c), within one column, indicate significant differences ($p < 0.05$) for 1 min of alkalization, while capital lowercase letters (A-C), within one column, indicate significant differences ($p < 0.05$) for 10 min of alkalization between alkali concentrations following one-way ANOVA and post hoc test.

n. d. non detected.

Strecker degradation (Belitz et al., 2009). The latter consists of a complex sequence of reactions producing pyrroles and pyridines, which on their turn lead to the formation of brown melanoidin pigments through polymerization (Aprotosoaie et al., 2016). Generally, a darker color in alkalized cocoa powder is desirable, since it gives the impression of intense cocoa flavors in food products. However, prolonged heating during alkalization might eventually be detrimental to the flavor (Moser, 2015). Our results are in accordance with Stanley et al. (2015) who reported a substantial increase of pH (5.57–8.03) and 13% decrease of L^* value in cocoa powder after half a minute of alkalization. It is very likely that this reaction even took place in a matter of seconds since the cocoa powder has a large surface contact area, due to its small particle size, which may cause an immediate reaction when brought into contact with an alkali agent.

Generally, both a^* and b^* values are relevant in order to calculate the a^*/b^* ratio. High a^*/b^* ratios (>1.6) correspond to intense reddish cocoa powders (Wiant et al., 1991). Based on our results, the a^*/b^* ratios of all alkalized cocoa powder were found in a rather narrow range of 0.29–0.56. These results were expected, as one of the limitations of cocoa powder alkalization is the narrow range of color change (Moser, 2015).

3.1.2. *Effect of alkali concentration*

The results of color and pH measurement of non-alkalized and alkalized cocoa powders while varying alkali concentration under a constant temperature of 90 °C are shown in Table 2. Compared to the previous experiment, the amount of alkali had a greater influence on the final pH values. Based on the classification of alkalized cocoa powders, the powders treated with NaOH 0.59 and 1.17% w/w fall into the lightly alkalized range (pH 6.5–7.2), while the ones treated with NaOH 2.34 and 3.59% w/w fall into the heavily alkalized range (pH > 7.6) irrespective of the alkalization time (Miller et al., 2008). Unlike the others, the pH values of powders treated with NaOH 0.59% w/w were below 7.2, which indicates that this alkali concentration was not sufficient to fully neutralize the acidity of the natural cocoa powder.

Similar to the previous experiment, the majority of color darkening (reduction of L^* value) occurred during the first minute (15–75%) of the alkalization process, followed by a small reduction of the L^* value (7-2%) as alkalization continued. In total, a wider range of L^* values was obtained through variation of the alkali concentration (15–82% reduction). A more intense dark color of cocoa powder was obtained with higher alkali concentrations (Stanley et al., 2015).

While no clear trend of a^* and b^* values was observed, a significant increase ($p < 0.05$) of a^*/b^* ratio with higher alkali concentrations was found. This implies that there was an increase of redness of the alkalized powders. It has been reported that large molecular red chromophores can be formed during alkalization, by reaction of polyphenols with catechinic acids, with hydroxyxanthene as the intermediate (Germann et al., 2019a, 2019b). Nevertheless, the values are still not in the range of deep red colored cocoa powders (Rodríguez et al., 2009; Wiant et al., 1991).

3.2. *Phytochemicals*

3.2.1. *Effect of alkalization temperature*

The HPLC-UV results in Table 3 show that alkalization did not significantly affect the theobromine concentration in cocoa powder ($p > 0.05$), while it reduced caffeine contents by 22–35% within the first min of alkalization. The exact mechanism of the effect of alkalization on methylxanthines in cocoa is not known yet. Our results suggest that the changes in methylxanthine content, particularly of caffeine, were primarily due to the alkaline environment created immediately after the addition of alkali, since the major decrease was found within the very first minute of alkalization, while little to no effect was observed with higher alkalization temperatures and longer time. However, further research is required to confirm this and to determine the mechanism behind methylxanthines. Caffeine and theobromine affect the sensory properties of cocoa powder, as they evoke a bitter taste (Afoakwa et al., 2008). Hence, a lower caffeine content in the alkalized samples might increase the palatability of cocoa powder (Hufnagel & Hofmann, 2008).

The flavan-3-ol content of cocoa powder was affected more by temperature compared to alkalization time, and major changes again occurred within the very first minute of the reaction at all temperatures. During the first minute of alkalization, catechin content rapidly increased by 42–181%, while conversely at the same time, epicatechin

Table 5

Semi-quantitative concentrations (μg 4-methylpyridine/g cocoa drink) of volatiles of non-alkalized and alkalized cocoa powders after 1 min of alkalization at constant alkali concentration of NaOH 2.4% w/w of cocoa powder and different alkalization temperatures (n = 3), and their odor description found in the literature.

Volatile compounds	Odor description[a]	Semi-quantitative concentration (eq. μg 4-methylpyridine/g cocoa powder)						KIexp	KIlit[b]
		Non-alkalized	60 °C	70 °C	80 °C	90 °C	100 °C		
Organic acids									
Acetic acid	Sour, vinegar	0.237 ± 0.083^{b}	< LOQ	0.0137 ± 0.0052^{a}	< LOQ	< LOQ	0.013 ± 0.012^{a}	1427	1450
Nonanoic acid	Green, fat	< LOQ	0.0121 ± 0.0048^{a}	0.0137 ± 0.0082^{a}	0.0085 ± 0.0040^{a}	0.0136 ± 0.0034^{a}	< LOQ	1981	2202
Sum		0.237 ± 0.083	0.0121 ± 0.0048	0.027 ± 0.010	0.0085 ± 0.0040	0.0136 ± 0.0034	0.013 ± 0.012		
Alcohols									
1-Octanol, 3,7-dimethyl-	Floral	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.0076 ± 0.0013^{a}	1092	1664
1-Octen-3-ol	Mushroom	< LOQ	< LOQ	0.0117 ± 0.0036^{a}	0.00994 ± 0.00040^{a}	0.0104 ± 0.0010^{a}	< LOQ	1434	1394
1-Hexanol, 2-ethyl-	Rose, green	0.221 ± 0.033^{b}	< LOQ	0.055 ± 0.042^{a}	< LOQ	0.00968 ± 0.00074^{a}	< LOQ	1472	1487
2-Nonanol	Citrus, fruity, lemon, grass	< LOQ	0.0122 ± 0.0021^{b}	< LOQ	< LOQ	< LOQ	0.00906 ± 0.00049^{a}	1500	1663–1666
1-Octanol	Chemical, metal, burnt	0.0153 ± 0.0017^{b}	0.00579 ± 0.00038^{a}	0.00597 ± 0.00084^{b}	0.00558 ± 0.00047^{a}	0.00641 ± 0.00080^{a}	< LOQ	1533	1388
Benzyl alcohol	Sweet, floral	0.0213 ± 0.0035^{b}	0.00821 ± 0.00024^{a}	0.00845 ± 0.00055^{a}	0.00787 ± 0.00034^{a}	0.00999 ± 0.00043^{a}	0.0101 ± 0.0018^{a}	1787	1865
Phenylethyl alcohol	Floral, honey	0.335 ± 0.054^{b}	0.1148 ± 0.0077^{a}	0.1115 ± 0.0088^{a}	0.1073 ± 0.0034^{a}	0.1201 ± 0.0072^{a}	0.121 ± 0.015^{a}	1814	1925
Sum		0.592 ± 0.063	0.1410 ± 0.0080	0.193 ± 0.043	0.1307 ± 0.0034	0.1566 ± 0.0074	0.148 ± 0.015		
Esters									
Acetic ester, methyl ester	Green	< LOQ	0.0163 ± 0.0020^{a}	0.0236 ± 0.0036^{b}	0.0260 ± 0.0027^{b}	0.0230 ± 0.0014^{b}	0.0220 ± 0.0026^{a,b}	873	828–864
Acetic acid, ethenyl ester	Sweet, fruity	0.097 ± 0.022^{a}	< LOQ	0.0167 ± 0.0021^{a}	0.0155 ± 0.0010^{a}	0.0153 ± 0.0011^{a}	< LOQ	964	878
Benzeneacetic acid, methyl ester	Sweet, honey, jasmine	0.0143 ± 0.0020^{c}	0.0161 ± 0.0011^{b}	0.0153 ± 0.0011^{b}	0.01520 ± 0.00051^{b}	0.0093 ± 0.0016^{a}	< LOQ	1697	1748–1761
Benzeneacetic acid, ethyl ester	Fruity, sweet	0.0610 ± 0.0081^{b}	0.00843 ± 0.00093^{a}	0.00758 ± 0.00033^{a}	0.00796 ± 0.00022^{a}	0.0729 ± 0.0125^{a}	< LOQ	1718	1775–1793
Acetic acid, 2-phenylethyl ester	Honey, floral	0.210 ± 0.017^{b}	0.0694 ± 0.0062^{a}	0.0600 ± 0.0035^{a}	0.0639 ± 0.0012^{a}	0.0200 ± 0.0037^{a}	0.0641 ± 0.0053^{a}	1742	1803–1821
Sum		0.200 ± 0.023	0.1102 ± 0.0067	0.1232 ± 0.0055	0.1285 ± 0.0032	0.141 ± 0.013	0.0861 ± 0.0059		
Terpenes									
Linalool	Floral, rose, sweet, green, citrus	0.0237 ± 0.0027^{b}	0.00860 ± 0.00051^{a}	0.00768 ± 0.00047^{a}	0.00799 ± 0.00013^{a}	0.0088 ± 0.0011^{a}	0.007520 ± 0.00013^{a}	1522	1537
Sum		0.0237 ± 0.0027	0.00860 ± 0.00051	0.00768 ± 0.00047	0.00799 ± 0.00013	0.0088 ± 0.0011	0.007520 ± 0.00013		
Ketones									
Acetone	Pungent	0.201 ± 0.045^{a}	0.190 ± 0.020^{a}	0.144 ± 0.036^{a}	0.144 ± 0.011^{a}	0.167 ± 0.018^{a}	< LOQ	868	813–821
2-Butanone	Fruity	0.035 ± 0.011^{b}	0.01191 ± 0.00036^{a}	0.0142 ± 0.0011^{a}	0.0161 ± 0.0021^{a}	0.0248 ± 0.0065^{a,b}	0.0237 ± 0.0010^{a,b}	909	917–950
2,3-Butanedione	Buttery	< LOQ	0.01808 ± 0.0038^{b}	< LOQ	< LOQ	< LOQ	0.0151 ± 0.0010^{a}	965	949–980
3-Penten-2-one	Fruity	< LOQ	0.01066 ± 0.00033^{a}	0.0117 ± 0.0013^{a}	0.01044 ± 0.00076^{a}	0.0113 ± 0.0010^{a}	0.01223 ± 0.00047^{a}	1105	973
2-Heptanone	Fruity, coconut, cheesy, floral	0.0394 ± 0.0045^{b}	0.0115 ± 0.0011^{a}	0.0137 ± 0.0019^{a}	0.01444 ± 0.00077^{a}	< LOQ	0.0135 ± 0.018^{a}	1166	1170
2-Nonanone	Fruity, fresh, sweet	0.0571 ± 0.0066^{b}	0.0166 ± 0.0015^{a}	0.01575 ± 0.00046^{a}	0.01754 ± 0.00011^{a}	0.0189 ± 0.0022^{a}	0.017421 ± 0.000091^{a}	1377	1388
Ethanone, 1-(2-furanyl)	Sweet, balsamic, coffee	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.01503 ± 0.00076^{a}	1478	1475–1512
Acetophenone	Floral	< LOQ	0.238 ± 0.021^{a}	0.268 ± 0.086^{a}	0.263 ± 0.048^{a}	0.255 ± 0.014^{a}	0.327 ± 0.148^{a}	1607	1600–1655
Sum		0.333 ± 0.047	0.497 ± 0.029	0.468 ± 0.093	0.466 ± 0.049	0.477 ± 0.024	0.423 ± 0.148		
Pyrazines									
Methylpyrazine	nutty, chocolate, cocoa, roasted nuts	0.141 ± 0.046^{b}	0.03851 ± 0.00057^{a}	0.0470 ± 0.0036^{a}	0.0431 ± 0.0019^{a}	0.04804 ± 0.00065^{a}	0.0476 ± 0.0026^{a}	1252	1251
2,5-Dimethylpyrazine	Cocoa, roasted nuts	0.273 ± 0.055^{b}	0.1260 ± 0.0033^{a}	0.139 ± 0.012^{a}	0.1316 ± 0.0051^{a}	0.1443 ± 0.0051^{a}	0.1439 ± 0.0083^{a}	1311	1290–1358
2,6-Dimethylpyrazine	Nutty, coffee, green	0.087 ± 0.019^{b}	0.0259 ± 0.0010^{a}	0.0283 ± 0.0036^{a}	0.02617 ± 0.00040^{a}	0.02942 ± 0.00089^{a}	0.02913 ± 0.00038^{a}	1317	1300–1370
Ethylpyrazine	Peanut butter, musty, nutty	< LOQ	0.023687 ± 0.000060^{a}	0.0275 ± 0.0030^{b}	0.02573 ± 0.00038^{a,b}	0.02728 ± 0.00063^{b}	0.0264 ± 0.0010^{a,b}	1322	1323–1343
2,3-Dimethylpyrazine	Caramel, cocoa							1335	1315–1344

(*continued on next page*)

Table 5 (*continued*)

Volatile compounds	Odor description[a]	Semi-quantitative concentration (eq. μg 4-methylpyridine/g cocoa powder)						KIexp	KIlit[b]
		Non-alkalized	60 °C	70 °C	80 °C	90 °C	100 °C		
		0.084 ± 0.019^{b}	0.02447 ± 0.00013^{a}	0.0267 ± 0.0023^{a}	0.0246 ± 0.0010^{a}	0.0265 ± 0.0010^{a}	0.0254 ± 0.0013^{a}		
2-Ethyl-6-methylpyrazine	Green, nuts, roasted	0.150 ± 0.029^{b}	0.04829 ± 0.00087^{a}	0.0518 ± 0.0040^{a}	0.0485 ± 0.0019^{a}	0.0540 ± 0.0026^{a}	0.04967 ± 0.00076^{a}	1374	1381–1415
2-Ethyl-5-methylpyrazine	Nutty, raw potato	0.414 ± 0.076^{b}	0.1420 ± 0.0056^{a}	0.146 ± 0.011^{a}	0.1383 ± 0.0053^{a}	0.1501 ± 0.0094^{a}	0.1338 ± 0.0074^{a}	1380	1386–1453
Trimethylpyrazine	Roasted, potato, must	0.414 ± 0.076^{b}	0.1799 ± 0.0064^{a}	0.177 ± 0.013^{a}	0.1652 ± 0.0051^{a}	0.1802 ± 0.0079^{a}	0.164 ± 0.012^{a}	1393	1395
3-Ethyl-2,5-dimethylpyrazine	Potato, roast	< LOQ	0.1172 ± 0.0035^{a}	0.116 ± 0.010^{a}	0.1107 ± 0.0028^{a}	0.1199 ± 0.0083^{a}	0.1078 ± 0.0040^{a}	1432	1408–1477
2,3-Dimethyl-5-ethylpyrazine	Burnt, green bean, popcorn	0.236 ± 0.036^{b}	0.1119 ± 0.0021^{a}	0.1052 ± 0.0093^{a}	0.0984 ± 0.0024^{a}	0.1067 ± 0.0087^{a}	0.0901 ± 0.0054^{a}	1447	1460
Tetramethylpyrazine	Cocoa, coffee, green, mocha, roast	0.592 ± 0.085^{b}	0.2208 ± 0.0087^{a}	0.211 ± 0.017^{a}	0.2029 ± 0.0068^{a}	0.223 ± 0.018^{a}	0.207 ± 0.014^{a}	1461	1438–1474
3,5-diethyl-2-methyl-pyrazine	Cocoa, chocolate, rum, sweet roasted	0.205 ± 0.026^{b}	0.0669 ± 0.0018^{a}	0.0639 ± 0.0064^{a}	0.0620 ± 0.0020^{a}	0.0678 ± 0.0062^{a}	0.0587 ± 0.0017^{a}	1477	1492–1503
2,3,5-Trimethyl-6-ethylpyrazine	Candy, sweet	0.202 ± 0.009^{b}	0.0730 ± 0.0021^{a}	0.0699 ± 0.0070^{a}	0.0669 ± 0.0015^{a}	0.0739 ± 0.0066^{a}	0.0649 ± 0.0024^{a}	1496	1491–1521
Pyrazine, 2,5-dimethyl-3-(2-methylpropyl)-	Nuts	0.1122 ± 0.0089^{b}	0.0387 ± 0.0019^{a}	0.0363 ± 0.0042^{a}	0.03535 ± 0.00085^{a}	0.0401 ± 0.0042^{a}	0.03450 ± 0.00093^{a}	1507	1506–1521
Sum		2.19 ± 0.17	1.237 ± 0.014	1.245 ± 0.033	1.179 ± 0.012	1.291 ± 0.027	1.183 ± 0.023		
<u>Aldehydes</u>									
2-Methylbutanal	Chocolate	0.329 ± 0.096^{b}	0.1220 ± 0.0062^{a}	0.1168 ± 0.0091^{a}	0.1132 ± 0.0059^{a}	0.1054 ± 0.0060^{a}	0.0958 ± 0.0040^{a}	917	912
3-Methylbutanal	Chocolate	0.64 ± 0.17^{b}	0.1955 ± 0.0050^{a}	0.178 ± 0.013^{a}	0.1683 ± 0.0087^{a}	0.1604 ± 0.0057^{a}	0.1399 ± 0.0077^{a}	920	910
Hexanal	Grass, tallow, fat	0.0278 ± 0.0070^{b}	0.00680 ± 0.00022^{a}	0.0079 ± 0.0031^{a}	0.00513 ± 0.00048^{a}	< LOQ	< LOQ	1061	1084
Heptanal	Fat, citrus, rancid	0.0158 ± 0.0032^{b}	< LOQ	0.00374 ± 0.00049^{a}	0.0043 ± 0.0021^{a}	< LOQ	< LOQ	734	1174
2-Isopropyl-5-methylhex-2-enal	Malty barley, roasted coffee	0.0558 ± 0.0071^{b}	0.0140 ± 0.0010^{a}	0.01390 ± 0.00066^{a}	0.01381 ± 0.00081^{a}	< LOQ	0.01268 ± 0.00049^{a}	1349	1373
2-Nonanal	Fat, citrus, green	0.0610 ± 0.0052^{a}	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	1061	1385
Benzaldehyde	Sweet, bitter, almond, cherry	2.91 ± 0.55^{b}	0.937 ± 0.022^{a}	0.899 ± 0.062^{a}	0.861 ± 0.023^{a}	0.935 ± 0.041^{a}	0.878 ± 0.088^{a}	1495	1495
Benzeneacetaldehyde	Floral, honey	2.85 ± 0.46^{b}	0.3222 ± 0.0094^{a}	< LOQ	0.278 ± 0.040^{a}	0.330 ± 0.036^{a}	< LOQ	1378	1592–1650
5-Methyl-2-Phenyl-2-hexenal	Cocoa	0.04595 ± 0.00054^{b}	0.01041 ± 0.00060^{a}	0.272 ± 0 .017^{a}	< LOQ	< LOQ	0.0100 ± 0.0019^{a}	1931	2060
Sum		6.94 ± 0.74	1.61 ± 0.025	1.491 ± 0.066	1.444 ± 0.048	1.530 ± 0.055	1.136 ± 0.089		

Note: All results represent the mean ± SD ($\bar{X}$ ±SD eq. μg 4-methylpyridine/g cocoa powder) of independent measurements ($n = 3$).
Different lowercase letters (a-b), within one row, indicate significant differences ($p < 0.05$) between alkalization temperatures following one-way ANOVA and post hoc test.
LOQ: limit of quantification.

[a] Odor descriptions derived from Aprotosoaie et al. (2016), Rottiers et al. (2019) & http://www.flavornet.org/flavornet.html.

[b] KI derived from Rottiers et al. (2019) & C20M on http://www.flavornet.org/flavornet.html & http://webbook.nist.gov.

content dramatically decreased by 34–81% (Table 3). Furthermore, these effects were larger at elevated temperatures, with a particularly significant increase of catechin contents at temperatures >60 °C. As reported in literature, possible epimerization of (−)-epicatechin to (−)-catechin might take place during alkalization under high temperature and basic pH conditions (Kofink et al., 2007). However, the applied HPLC method was not capable of distinguishing the enantiomers, and thus, it was not possible to determine if, and how much (−)-catechin was formed.

Concomitantly with the epimerization reaction, both catechin and epicatechin might be degraded as well during alkalization. Polyphenols are prone to heat degradation with epicatechin reported to be more sensitive than catechin (Aprotosoaie et al., 2016). Under alkaline conditions, oxidation and subsequent polymerization of polyphenols might occur (Li et al., 2014). The high loss of flavan-3-ols is one of the undesirable aspects of cocoa alkalization, given the potential health benefits of these compounds. Moreover, epimerization from (−)-epicatechin to (−)-catechin can reduce the bioavailability of cocoa polyphenols due to the much higher bioavailability of epicatechin compared to catechin (Donovan et al., 2006; Rusconi & Conti, 2010). Furthermore, loss of epicatechin and possible degradation of proanthocyanidins cause a reduction in astringency and bitterness of the cocoa powder. This reduction in astringency and bitterness, as well as the reduction in acidity (rise in pH), can result in an improved flavor profile of alkalized cocoa powder compared to non-alkalized cocoa powder.

3.2.2. *Effect of alkali concentration*

The effect of different alkali concentrations on the levels of theobromine, caffeine, epicatechin, and catechin are presented in Table 4. A decreasing trend of theobromine was observed especially with the most intense reduction at 10 min of alkalization at the two highest alkali concentrations (2.34 and 3.59% w/w NaOH), however it was not significant. On the other hand, powder which was alkalized using higher alkali concentrations (2.34 and 3.59% w/w NaOH) seemed to have a significant lower caffeine content. This outcome suggests that caffeine degradation during alkalization might be dependent from the alkali

Table 6

Semi-quantitative concentrations (μg 4-methylpyridine/g cocoa drink) of volatiles of non-alkalized and alkalized cocoa powders after 1 min of alkalization at constant temperature of 90 °C and different alkali concentrations (n = 3), and their odor description found in the literature.

Volatile compounds	Odor description[a]	Semi-quantitative concentration (eq. μg 4-methylpyridine/g cocoa powder)						
		Non-alkalized	0.59% w/w NaOH	1.17% w/w NaOH	2.34% w/w NaOH	3.59% w/w NaOH	KIexp	KIlit[b]
Organic acids								
Acetic acid	Sour, vinegar	0.237 ± 0.083^{a}	0.0319 ± 0.0014^{b}	< LOQ	< LOQ	< LOQ	1427	1450
Octanoic acid	Sweet cheese	< LOQ	< LOQ	< LOQ	0.00667 ± 0.00058^{a}	< LOQ	1916	2083
Nonanoic acid	Green, fat	< LOQ	< LOQ	< LOQ	0.0240 ± 0.0014^{a}	< LOQ	1981	2202
Sum		0.237 ± 0.083	0.0319 ± 0.0014	< LOQ	0.0307 ± 0.0014	< LOQ		
Alcohols								
1-Octen-3-ol	Floral	< LOQ	< LOQ	< LOQ	0.00949 ± 0.00034^{a}	< LOQ	1434	1394
1-Hexanol, 2-ethyl-	Rose, green	0.221 ± 0.033^{a}	< LOQ	< LOQ	< LOQ	< LOQ	1472	1487
1-Octanol	Chemical, metal, burnt	0.0153 ± 0.0017^{b}	< LOQ	< LOQ	0.00575 ± 0.00086^{a}	0.00565 ± 0.00047^{a}	1533	1388
Benzyl alcohol	Sweet, floral	0.0213 ± 0.0035^{d}	0.0190 ± 0.0015^{c,d}	0.01453 ± 0.00047^{b,c}	0.00806 ± 0.00058^{a}	0.01218 ± 0.00032^{a,b}	1787	1865
2-Phenylethyl alcohol	Floral, honey	0.335 ± 0.054^{c}	0.207 ± 0.010^{b}	0.1528 ± 0.0075^{a,b}	0.118 ± 0.013^{a}	0.1321 ± 0.0054^{a}	1814	1925
Sum		0.592 ± 0.063	0.226 ± 0.010	0.1673 ± 0.0075	0.141 ± 0.013	0.1499 ± 0.0055		
Esters								
Acetic ester, methyl ester	Green	< LOQ	< LOQ	< LOQ	0.0253 ± 0.0057^{a}	0.0641 ± 0.0046^{b}	873	828–864
Acetic acid, ethenyl ester	Sweet, fruity	0.097 ± 0.022^{a}	0.0815 ± 0.0018^{c}	0.04329 ± 0.0081^{b}	0.01668 ± 0.00042^{a}	< LOQ	964	878
Benzoic acid, methyl ester	Herb, lettuce, prune, violet	< LOQ	< LOQ	< LOQ	0.00506 ± 0.00035^{a}	< LOQ	1581	1573–1640
Benzeneacetic acid, methyl ester	Sweet, honey, jasmine	0.0143 ± 0.0020^{c}	< LOQ	0.0185 ± 0.0023^{b}	0.0156 ± 0.0017^{b}	< LOQ	1697	1748–1761
Benzeneacetic acid, ethyl ester	Fruity, sweet	0.0610 ± 0.0081^{d}	0.0131 ± 0.0010^{c}	0.0098 ± 0.0012^{b,c}	0.0087 ± 0.0010^{b}	< LOQ	1718	1775–1793
Acetic acid, 2-phenylethyl ester	Honey, floral	0.210 ± 0.017^{c}	0.1030 ± 0.0093^{b}	0.0788 ± 0.0086^{a,b}	0.069 ± 0.010^{a}	0.0543 ± 0.0023^{a}	1742	1803–1821
Sum		0.200 ± 0.023	0.198 ± 0.010	0.1503 ± 0.0090	0.141 ± 0.012	0.1183 ± 0.0051		
Terpenes								
Linalool	Floral, rose, sweet, green, citrus	0.0237 ± 0.0027^{b}	0.00995 ± 0.00069^{a}	0.00794 ± 0.00043^{a}	0.00774 ± 0.00073^{a}	0.007078 ± 0.000085^{a}	1522	1537
Sum		0.0237 ± 0.0027	0.00995 ± 0.00069	0.00794 ± 0.00043	0.00774 ± 0.00073	0.007078 ± 0.000085		
Ketones								
Acetone	Pungent	0.201 ± 0.045^{b}	0.459 ± 0.045^{a}	0.220 ± 0.040^{a}	0.134 ± 0.042^{a}	0.11 ± 0.10^{a}	868	813–821
2-Butanone	Fruity	0.035 ± 0.011^{b}	0.0194 ± 0.0016^{a}	0.0162 ± 0.0014^{a}	0.0142 ± 0.011^{a}	0.03450 ± 0.00052^{b}	909	917–950
2,3-Butanedione	Buttery	< LOQ	< LOQ	0.04329 ± 0.00081^{a}	< LOQ	< LOQ	965	949–980
3-Penten-2-one	Fruity	< LOQ	< LOQ	0.0093 ± 0.0013^{a}	0.01305 ± 0.00055^{b}	0.00955 ± 0.00040^{a}	1105	973
2-Heptanone	Fruity, coconut, cheesy, floral	0.0394 ± 0.0045^{b}	0.0137 ± 0.0039^{a}	< LOQ	0.0147 ± 0.0024^{a}	0.0158 ± 0.0018^{a}	1166	1170
2-Nonanone	Fruity, fresh, sweet	0.0571 ± 0.0066^{b}	0.0173 ± 0.0018^{a}	0.01303 ± 0.00065^{a}	0.0184 ± 0.0017^{a}	0.0148 ± 0.0013^{a}	1377	1388
Ethanone, 1-(2-furanyl)	Sweet, balsamic, coffee	< LOQ	< LOQ	< LOQ	< LOQ	0.0193 ± 0.0016^{a}	1478	1475–1512
Acetophenone	Floral	< LOQ	< LOQ	< LOQ	0.2960 ± 0.0067^{a}	< LOQ	1607	1600–1655
1-Propanone, 1-phenyl	Fruity	< LOQ	0.00647 ± 0.00037^{a}	< LOQ	< LOQ	0.00853 ± 0.00065^{b}	1667	1682–1734
Sum		0.333 ± 0.047	0.600 ± 0.045	0.364 ± 0.040	0.490 ± 0.043	0.21 ± 0.10		
Pyrazines								
Methylpyrazine	Nutty, chocolate, cocoa, roasted nuts	0.141 ± 0.046^{b}	0.0702 ± 0.0012^{a}	0.0460 ± 0.0056^{a}	0.0507 ± 0.0019^{a}	0.0500 ± 0.0026^{a}	1252	1251
2,5-Dimethylpyrazine	Cocoa, roasted nuts	0.273 ± 0.055^{b}	0.1537 ± 0.0026^{a}	0.124 ± 0.012^{a}	0.1454 ± 0.0047^{a}	0.1451 ± 0.0082^{a}	1311	1290–1358
2,6-Dimethylpyrazine	Nutty, coffee, green	0.087 ± 0.019^{b}	0.0455 ± 0.0015^{a}	< LOQ	0.0301 ± 0.0012^{a}	0.0315 ± 0.0019^{a}	1317	1300–1370
Ethylpyrazine	Peanut butter, musty, nutty	< LOQ	0.0434 ± 0.0014^{b}	0.0292 ± 0.0024^{a}	0.0295 ± 0.0015^{a}	0.0285 ± 0.0023^{a}	1322	1323–1343

(*continued on next page*)

Table 6 (*continued*)

Volatile compounds	Odor description[a]	Semi-quantitative concentration (eq. μg 4-methylpyridine/g cocoa powder)						
		Non-alkalized	0.59% w/w NaOH	1.17% w/w NaOH	2.34% w/w NaOH	3.59% w/w NaOH	KIexp	KIlit[b]
2,3-Dimethylpyrazine	Caramel, cocoa	0.084 ± 0.019^{b}	0.0441 ± 0.0012^{a}	0.0306 ± 0.0016^{a}	0.02735 ± 0.00075^{a}	0.0276 ± 0.0012^{a}	1335	1315–1344
2-Ethyl-6-methylpyrazine	Green, nuts, roasted	0.150 ± 0.029^{b}	0.0758 ± 0.0025^{a}	0.0547 ± 0.0033^{a}	0.0540 ± 0.0031^{a}	0.0577 ± 0.0027^{a}	1374	1381–1415
2-Ethyl-5-methylpyrazine	Nutty, raw potato	0.414 ± 0.076^{a}	0.1355 ± 0.0063^{c}	0.1098 ± 0.0097^{b}	< LOQ	0.1507 ± 0.0074^{c}	1380	1386–1453
Trimethylpyrazine	Roasted, potato, must	0.414 ± 0.076^{b}	0.2299 ± 0.0083^{a}	0.1769 ± 0.0093^{a}	0.162 ± 0.012^{a}	0.1889 ± 0.0082^{a}	1393	1395
3-Ethyl-2,5-dimethylpyrazine	Potato, roast	< LOQ	0.1187 ± 0.0069^{a}	0.1337 ± 0.0084^{a}	0.1160 ± 0.0099^{a}	0.1222 ± 0.0041^{a}	1432	1408–1477
2,3-Dimethyl-5-ethylpyrazine	Burnt, green bean, popcorn	0.236 ± 0.036^{b}	0.0886 ± 0.0078^{a}	0.0952 ± 0.0067^{a}	0.0992 ± 0.0086^{a}	0.1117 ± 0.0041^{a}	1447	1460
Tetramethylpyrazine	Cocoa, coffee, green, mocha, roast	0.592 ± 0.085^{c}	0.1019 ± 0.0084^{a}	$0.256 \pm 0.015^{a,b}$	0.214 ± 0.019^{b}	$0.2352 \pm 0.0051^{a,b}$	1461	1438–1474
3,5-diethyl-2-methyl-pyrazine	Cocoa, chocolate, rum, sweet roasted	0.205 ± 0.026^{b}	0.0491 ± 0.0046^{a}	0.0706 ± 0.0052^{a}	0.0636 ± 0.0063^{a}	0.0646 ± 0.0030^{a}	1477	1492–1503
2,3,5-Trimethyl-6-ethylpyrazine	Candy, sweet	0.202 ± 0.009^{b}	0.1019 ± 0.0084^{a}	0.0797 ± 0.0062^{a}	0.0692 ± 0.0072^{a}	0.0726 ± 0.0016^{a}	1496	1491–1521
Pyrazine, 2,5-dimethyl-3-(2-methylpropyl)-	Nuts	0.1122 ± 0.0089^{b}	0.0491 ± 0.0046^{a}	0.0420 ± 0.0039^{a}	0.0367 ± 0.0045^{a}	0.0368 ± 0.0016^{a}	1507	1506–1521
Sum		2.91 ± 0.17	1.307 ± 0.020	1.249 ± 0.028	1.097 ± 0.029	1.323 ± 0.017		
Aldehydes								
2-Methylbutanal	Chocolate	0.329 ± 0.096^{b}	0.117 ± 0.010^{a}	0.0982 ± 0.0078^{a}	0.1162 ± 0.0051^{a}	0.2553 ± 0.0052^{b}	917	912
3-Methylbutanal	Chocolate	0.64 ± 0.17^{c}	$0.2333 \pm 0.0079^{a,b}$	$0.1709 \pm 0.0060^{a,b}$	0.1618 ± 0.0078^{a}	0.3689 ± 0.0068^{b}	920	910
Hexanal	Grass, tallow, fat	0.0278 ± 0.0070^{b}	< LOQ	0.00676 ± 0.00044^{a}	0.00551 ± 0.00020^{a}	< LOQ	1061	1084
Heptanal	Fat, citrus, rancid	0.0158 ± 0.0032^{b}	0.01177 ± 0.00092^{a}	< LOQ	< LOQ	< LOQ	734	1174
2-Isopropyl-5-methylhex-2-enal	Malty barley, roasted coffee	0.0558 ± 0.0071^{b}	< LOQ	0.00954 ± 0.00060^{a}	0.01306 ± 0.00083^{a}	0.01045 ± 0.0089^{a}	1349	1373
2-Nonanal	Fat, citrus, green	0.0610 ± 0.0052^{c}	0.0303 ± 0.0042^{b}	$0.0222 \pm 0.0045^{a,b}$	0.0162 ± 0.0016^{a}	< LOQ	1061	1385
Benzaldehyde	Sweet, bitter, almond, cherry	2.91 ± 0.55^{c}	2.031 ± 0.092^{b}	1.514 ± 0.075^{b}	0.734 ± 0.0037^{a}	1.495 ± 0.071^{b}	1495	1495
Benzeneacetaldehyde	Floral, honey	2.85 ± 0.46^{b}	0.689 ± 0.099^{a}	0.427 ± 0.071^{a}	0.240 ± 0.028^{a}	0.242 ± 0.035^{a}	1378	1592–1650
5-Methyl-2-Phenyl-2-hexenal	Cocoa	0.04595 ± 0.00054^{c}	0.0178 ± 0.0026^{b}	0.0144 ± 0.0015^{b}	0.0099 ± 0.0017^{a}	0.00728 ± 0.00045^{a}	1931	2060
Sum		6.94 ± 0.74	3.13 ± 0.14	2.24 ± 0.10	1.297 ± 0.047	2.379 ± 0.080		

Note: All results represent the mean ± SD ($\overline{X}$ ±SD eq. μg 4-methylpyridine/g cocoa powder) of independent measurements ($n = 3$).
Different lowercase letters (a–d), within one row, indicate significant differences ($p < 0.05$) between alkali concentrations following one-way ANOVA and post hoc test.
LOQ: limit of quantification.

[a] Odor descriptions derived from Aprotosoaie et al. (2016), Rottiers et al. (2019) & http://www.flavornet.org/flavornet.html.

[b] KIlit derived from Rottiers et al. (2019) & C20M on http://www.flavornet.org/flavornet.html & webbook.nist.gov.

concentration. A possible explanation for its degradation is the occurrence of an alkaline hydrolysis reaction, resulting in the formation of caffeidine (Kumar et al., 1993). However, further research is required to determine the mechanism of the effect of alkalization on methylxanthines.

By varying the alkali concentration, the level of epicatechin could be affected to a higher extent (11–93%) under constant temperature of 90 °C. Bigger losses were obtained at higher alkali concentrations, which is in line with literature (Li et al., 2012). The concentration of epicatechin of cocoa powder alkalized with 3.59% w/w NaOH for 10 min was lower than the limit of detection (LOD) of the analysis method (0.2 mg/g defatted powder). On the other hand, non-linear behavior was observed for catechin in the alkalized cocoa powder, which peaked at 1.17% w/w NaOH when alkalized for 10 min. This might be due to a larger extent of degradation compared to production (through epimerization) of catechin at higher alkali concentrations.

3.3. Volatile compounds

Since the major changes of pH, color, flavan-3-ols and caffeine were observed during the first minute of alkalization, HS-SPME-GC-MS was performed in cocoa drinks of 1 min alkalization. It was found that the alkalization process clearly affected the aromatic compounds. Results shown in Tables 5 and 6 revealed that alkalization positively affected the average total of measured ketones (except for the sample alkalized with NaOH 3.59% w/w). This might result from accelerated oxidation of alcohols to ketones when applying an alkali treatment. As reported in the study of Huang and Barringer (2010) and the review of Aprotosoaie et al. (2016), alkalization results in a more intense aroma profile. However, results reported in this study showed large reductions in concentration, for most of the chemical classes of volatile compounds (organic acids, alcohols, esters, terpenes, pyrazines and aldehydes). Several processes might have contributed to this effect. Firstly, highly volatile aromatic compounds, mainly formed during roasting, are likely prone to evaporation during the alkalization process. Secondly, the depletion of precursors, which were consumed during this prior roasting step, might have resulted in the absence of additional aroma formation during the alkalization. These effects could probably be avoided partially by applying the alkalization treatment before the roasting step. This hypothesis was confirmed by Huang and Barringer (2010), who reported that alkalization resulted in a more intense aroma when applied prior to roasting instead of vice versa. Another possible factor causing a decrease of volatile compounds was suggested by Li et al. (2012), who stated that alkaline conditions favor potential interactions between polyphenols and aroma molecules, impeding the latter to evaporate.

3.3.1. *Effect of alkalization temperature*

As shown in Table 5, the impact of temperature on the aroma profile during alkalization was rather limited in the studied range (60–100 °C). Normally, the formation of volatile compounds resulting from the Maillard reaction are favored at higher temperatures, especially above 100 °C. Nevertheless, as stated in Huang and Barringer (2010) at alkaline conditions, temperatures of less than 100 °C would be sufficient to promote the Maillard reaction. The insignificant differences between the semi-quantified volatiles of the different treatments, could possibly be explained by a too narrow range of the selected temperatures, or because of the order of the applied processes. The effect of using milder temperatures during the subsequent alkalization treatment using commercial cocoa powder, which previously underwent a roasting treatment, might be rather negligible. Also, the absence of significant differences for semi-quantified pyrazines (except ethylpyrazine) and aldehydes might be attributed to the depletion of precursors of these volatiles, caused by the preceding roasting step. For chemical classes, such as organic acids, alcohols, esters and terpenes, a clear reduction was measured between the non-alkalized sample and samples alkalized at 60 °C. However, no substantial additional reduction took place by further increasing the temperature to 100 °C.

3.3.2. *Effect of alkali concentration*

Contrary to the effect of varying temperatures, the impact of varying NaOH concentrations on the aroma profile was more pronounced for certain chemical classes in the studied range (Table 6). Acetic acid, the dominant organic acid, was already reduced more than 85% when alkalizing with NaOH 0.59% w/w. A further decrease resulted in detected concentrations below the LOQ. Likely, a higher NaOH concentration resulted in neutralization of this acid, to form its corresponding salt (sodium acetate) and water. Generally, a clear decreasing trend was observed for the total average of the detected alcohols and esters with increasing concentrations of NaOH. It might thus be concluded that a more intense alkalization potentially results in less fruity and floral aromas. Previously, contradictory results were reported for the effect of applied alkali concentration on alcohol levels. While Li et al. (2012) observed that an alkalization treatment of 1% or 3% NaOH did not have a significant impact on the level of alcohols, Huang and Barringer (2010) reported an increase of alcohols at higher pH values. Nevertheless, this research showed significantly ($p < 0.05$) decreased levels for both benzyl- and 2-phenylethyl alcohol when intensifying the alkali treatment from 0.59% to 3.59% w/w. An increased oxidation of these alcohols to their respective aldehydes (benzaldehyde and 2-phenylacetaldehyde) and organic acids (benzoic acid and 2-phenylacetic acid) could be the reason for this decrease. However, this was not reflected in the measured aldehyde and organic acid levels, possibly due to a higher extent of evaporation of these molecules, compared to their formation. Alcohols could also have been formed during alkalization, by the saponification reaction of NaOH with esters. These reactions can explain the larger decrease of esters when alkalizing at higher NaOH concentrations. According to Li et al. (2012) the total average of measured ketones decreased when increasing the alkali concentration; however, no explanation was given in this study. Possibly these ketones, together with aldehydes, were consumed as precursors in the formation of heterocyclic compounds such as pyrazines. As stated in the research of Alasti et al. (Alasti et al., 2020), a higher pH results in an enhanced Maillard reaction and consequently an increased alkylpyrazine formation. Nevertheless, no significant increase was measured in pyrazine concentration at higher NaOH levels. This phenomenon could be explained by the hypothesis of Li et al. (2012) who assumed that at higher pH levels, pyrazines and their precursors could bind with polyphenols.

4. Conclusions

The results of this study indicate that most of the changes in pH, color, flavan-3-ols and caffeine concentration, take place in the first minute of alkalization. High alkali concentrations do not benefit elevated levels of the phytochemicals. The fast reaction of alkalization seems to favor the degradation of epicatechin. While the impact of varying the temperature on the aroma profile was limited, it was more pronounced for certain chemical classes of compounds when alkalizing at higher NaOH concentrations. Probably, a clearer effect of temperature on volatiles could have been observed if alkalization was performed prior the roasting treatment or when broadening the temperature range. Although the rapid process might reduce the production cost of alkalized cocoa powder, the quick occurrence of changes might be a disadvantage, as, it might be difficult to precisely control the process, compared to other types of cocoa alkalization.

Notes

The authors declare no competing financial interest.

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