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Original Research Article

Reduction of soluble oxalate in cocoa powder by the addition of calcium and ultrasonication

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ABSTRACT

Cocoa bean and its products are high-value, nutritious foods, but also contain considerably amounts of soluble oxalate that is linked with increased incidences of forming kidney stones. This study investigated the potential of reducing soluble oxalate contents in cocoa powder by mixing with three calcium salts (calcium carbonate $CaCO_3$, calcium chloride dihydrate $CaCl_2.2H_2O$ and calcium sulfate dihydrate $CaSO_4.2H_2O$), three milk varieties (full-cream, low-fat and high-calcium milk) and ultrasonication at different combinations of temperatures (40, 60 and 80 °C) and time (1–6 h). Analyses of soluble oxalate were carried out using Ultra High-Performance Liquid Chromatography (UHPLC). Among six calcium sources, $CaSO_4.2H_2O$ and high-calcium milk were the most effective treatments that reduced 31.78 % and 21.14 % soluble oxalate in cocoa powder, respectively. Ultrasonication reduced up to 39.96 % soluble oxalate in cocoa powder, but the efficiencies greatly depended on temperatures, durations and the interactive effects between the two factors. It was concluded that the addition of calcium using its salts or milk products and ultrasonication were potential methods to reduce soluble oxalate and make cocoa powder safer for consumers at risks of hyperoxaluria.

1. Introduction

Oxalic acid is a naturally occurring substance found in many plant foods such as spinach, beetroot, rhubarb (Siener et al., 2006), cocoa (Nguyễn et al., 2018), coffee, nuts, tea and taro (Noonan and Savage, 1999). Oxalates can be present in soluble form (i.e. sodium and potassium salts or free oxalic acid) and insoluble form (i.e. calcium and magnesium salts) (Noonan and Savage, 1999). The consumption of oxalate-rich foods, particularly those high in soluble oxalate, could increase the risks of hyperoxaluria (excessive excretion of oxalate in urine) and calcium deficiency. Under supersaturated conditions, oxalate in urine tended to be crystallized and form kidney stones, mainly calcium oxalate (Massey, 2007). In addition, soluble oxalate can combine with calcium to form insoluble salts at neutral or alkaline pH as in the small intestine (Noonan and Savage, 1999), rendering calcium ion unavailable for absorption (Libert and Franceschi, 1987).

Cocoa (*Theobroma cacao* L.) and cocoa products are now leading health food markets owing to their unique flavors and health benefits (Rusconi and Conti, 2010), but their potentially harmful effects due to containing oxalates are usually neglected. Consumers, apart from people suffering kidney stones, are not fully aware of the soluble oxalate contents in cocoa products. The mean total oxalate content of cocoa powder produced from four maturity stages of cocoa beans was 632 ± 20 mg/100 g dry matter (DM) of which soluble oxalates were 89 % (Nguyễn et al., 2018). Analyses of fifteen commercial products of cocoa powders from four countries revealed soluble oxalate contents ranging from 360 to 567 mg/100 g DM (Schroder et al., 2011). Higher soluble oxalate contents were recently reported in several samples of conventionally produced cocoa, ranging from 727.5 to 1477.5 mg/100 g DM (Witt et al., 2016).

As the incidence of having kidney stones has been increasing worldwide (Romero et al., 2010), efforts have been made to investigate the impacts of processing on oxalate contents of cocoa powder (Nguyễn et al., 2018; Schroder et al., 2011). However, reduction of oxalates in this food have not been taken into account. Trials to degrade pure oxalic acid by ultrasound obtained potential results (Dükkanci et al., 2006), but there have not been studies whether the same effect would be achieved in more complex matrices such as food. Dietary oxalate has been shown to make up of a large proportion of renal oxalates, normally ~50 % (Holmes et al., 2001; Liebman and Chai, 1997), but higher values up to 67 % have also been reported (Holmes et al., 1995). Therefore, reducing oxalate availability in food such as cocoa products would be necessary to make cocoa-based products safe for consumers. This study aimed to trial two separated approaches for reducing the soluble oxalate in cocoa powders: by adding calcium in forms of salts ($CaCO_3$; $CaCl_2.2H_2O$ and $CaSO_4.2H_2O$) and milk (full-cream, low-fat

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and high-calcium milk), or by ultrasonication at different temperatures (40, 60 and 80 °C) and time (1–6 h). This is the first time, impacts of these factors on oxalate reduction of cocoa powder have been investigated.

2. Materials and methods

2.1. Preparation of cocoa powder

Half-ripe cocoa fruits (TD6 genotype) were harvested in Ben Tre province, Vietnam. After removed from placentas, cocoa beans were naturally fermented in wooden boxes for 5 days and sun-dried on mats until moisture contents were lower than 10 % (Nguyễn et al., 2018). Dried cocoa beans were further processed through roasting, grinding, pressing and pulverizing at Lam Anh Cocoa Private Company (Tien Giang, Vietnam) to obtain fine cocoa powders. Samples were prepared in triplicate and stored at –20 °C until used.

2.2. Study on calcium sources

The soluble oxalate content of cocoa powder was determined at the beginning of the experiment. To study the oxalate binding capacity of the calcium salts, $CaCO_3$, $CaCl_2.2H_2O$ and $CaSO_4.2H_2O$ (Merck, Darmstadt, Germany) were added to 0.1 g cocoa powder in 100 mL nanopure water (Barnstead EasyPure II, Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, MA, USA), as it was assumed that one Ca^{+2} would bind with one $(COO^-)_2$:

$$m_{Ca\ salt} = M_{Ca\ salt} \times \frac{m_{\ oxalate\ in\ 0.1\ g\ cocoa}}{M_{(COO^-)_2}} = M_{Ca\ salt} \times \frac{m_{\ oxalate\ in\ 0.1\ g\ cocoa}}{88}$$

In the experiment with milk as the calcium sources, 0.1 g cocoa powder was mixed with 100 mL of milk (full-cream, low-fat and high-calcium). The calcium contents of the full-cream, low-fat and high-calcium milk were provided by the Meadow Fresh, New Zealand (Table 1). Samples were incubated at 30 °C, 200 rpm for 1 h in a shaking incubator (KS 4000 ic control, IKA, Wilmington, NC, USA).

2.3. Study on ultrasonication

Sonication of cocoa samples was carried out indirectly using an ultrasonic bath (WUC-A10H, Daihan-Brand, Korea). In detail, 250 mL Erlenmeyer flasks with an approximate diameter of 82 mm and a uniform wall thickness of 5 mm were used to contain mixtures of 0.1 g cocoa in 100 mL nanopure water. The flasks were then immersed in the ultrasonic bath containing 3 L of water as the coupling fluid. The operating frequency and power of the bath were fixed at 40 kHz and 265 W, whereas temperatures (40, 60 and 80 °C) and time (1–6 h) were adjusted to desired values. Sonicated samples were stored in refrigerator (4 °C) until analyses within a week.

2.4. Determination of soluble oxalate contents

Soluble oxalate contents were analysed following the method of Huynh and Nguyen (2017) with minor modifications. Briefly, soluble oxalate was extracted by adding 0.01 g of untreated cocoa powders with 100 mL of nanopure water, or 10 mL of well-mixed calcium-added or sonicated cocoa samples (equivalent to 0.01 g cocoa powder) with 90 mL of nanopure water. All the mixtures were kept at 80 °C, 200 rpm for 20 min in the shaking incubator. The oxalate extracts were cooled to room temperature, centrifuged at 6000 rcf for 20 min and filtered through 0.45 µm cellulose acetate filters (Membrane Solution, USA) before UHPLC analysis (Dionex Ultimate 3000 HPLC System, USA).

Chromatographic analysis was performed on a 300 × 7.8 mm Rezex ROA ion exclusion organic acid column (Phenomenex, USA) with an isocratic flow of sulfuric acid 25 mmol/L (Merck, USA) at 0.4 mL/min. The system was equipped with a UV/VIS detector set at 210 nm to capture absorbance every 0.02 min. Results were compared against oxalic acid standards and presented as mean values ± standard deviation of mg oxalate/100 g cocoa powder. All determinations were performed in triplicate.

Table 1
Calcium and fat contents in three milk products (stated by Meadow Fresh, NZ).

Milk product	Calcium content (mg/100 mL)	Fat content (g/100 mL)
Full-cream milk	120	3.3
Low-fat milk	125	1.5
High-calcium milk	181	1.4

2.5. Statistical analysis

One-way ANOVA with Fisher's post-hoc test at a significance level of $p < 0.05$ was used to study the effects of different calcium and ultrasound treatments on soluble oxalate contents in cocoa powder (Minitab 18, Minitab Inc., State College, PA, USA). The interaction between temperature and time in ultrasonic treatments on those measurements was studied using two-way ANOVA at $p < 0.05$.

2.6. Recovery study

The recovery of oxalic acid was examined by adding 5 mg of oxalic acid to 0.05 g of cocoa powder in 100 mL nanopure water, followed by the extraction and quantification of soluble oxalate as other samples. The average recovery of the added oxalic acid was 92.05 ± 2.41 %.

3. Results and discussion

3.1. Effects of different calcium sources on soluble oxalate contents in cocoa powder

Overall, the addition of calcium salts and milk reduced soluble oxalate contents in cocoa powder with an average of 15.51 % reduction found in calcium salts – cocoa mixtures, compared to 13.26 % in the milk – cocoa mixtures (Table 2). However, the oxalate binding capacity varied greatly among the calcium sources. $CaSO_4.2H_2O$ and high-calcium milk were the best treatments that reduced 31.78 % and 21.14 % of cocoa soluble oxalate, respectively. In contrast, full-cream milk and $CaCO_3$ were the least suitable calcium sources for reducing soluble oxalate in cocoa with only 5.73–5.80% reduction.

The reduction of soluble oxalate in cocoa powder observed in this study could be resulted from the binding of oxalates by the calcium ions available in the soluble fractions of the mixtures. $CaCl_2.2H_2O$ is highly soluble, so theoretically, should have provided sufficient Ca^{2+} to bind the soluble oxalate from cocoa powder. However, $CaCl_2.2H_2O$ in this experiment only reduced 8.95 % of soluble oxalate (Table 2), suggesting the formation of oxalate complexes and interactions between components existing in the food matrices. Indeed, increase in the solubility of calcium oxalate was observed at high concentrations of Ca^{2+} (> 10 mM) when adding $CaCl_2$ into an aqueous 0.134 M NaCl solution in equilibrium with calcium oxalate monohydrate (Finlayson and Roth, 1973). It was concluded that even in simple solutions, there were already at least three types of oxalate complexes, namely calcium oxalate, dicalcium oxalate and monosodium oxalate. For sparingly soluble salts like $CaCO_3$ and $CaSO_4$, similar effects are unlikely to occur due to the lower concentrations of Ca^{2+} available. Instead, the addition of $CaCO_3$ or $CaSO_4$ would shift the equilibrium of the oxalate-containing medium towards the formation of the less soluble salts. Because the solubility of $CaCO_3$ < calcium oxalate < $CaSO_4$, $CaSO_4$-added samples would result in more oxalate binding than the CO_3-added ones, which matched with findings in the current study (Table 3).

Among three types of milk used, high-calcium and low-fat milk had

Table 2
Effects of six calcium sources on the reduction of soluble oxalate in cocoa powder.

Calcium source	Initial soluble oxalate (mg/100 g DM)	Soluble oxalate after reaction (mg/100 g DM)	Reduced soluble oxalate (mg/100 g DM)	% Reduction of soluble oxalate
$CaCO_3$	616.05 ± 0.45	580.34 ± 1.06^a	35.71 ± 1.20^e	5.80 ± 0.19^e
$CaCl_2.2H_2O$	616.05 ± 0.45	560.93 ± 1.77^b	55.12 ± 1.64^d	8.95 ± 0.27^d
$CaSO_4.2H_2O$	616.05 ± 0.45	420.28 ± 1.01^e	195.78 ± 1.18^a	31.78 ± 0.18^a
Full-cream milk	616.05 ± 0.45	580.75 ± 2.86^a	35.31 ± 2.56^e	5.73 ± 0.42^e
Low-fat milk	616.05 ± 0.45	536.46 ± 1.65^c	79.59 ± 1.58^c	12.92 ± 0.26^c
High-calcium milk	616.05 ± 0.45	485.83 ± 3.06^d	130.23 ± 2.83^b	21.14 ± 0.47^b

Means with different letters in each column are significantly different ($p < 0.001$).

the greater effects on lowering soluble oxalate contents in cocoa powder, compared to full-cream milk owing to its higher calcium content and less fat (Table 1). The presence of fatty acids was suggested to hinder bindings between calcium and oxalate (Bailly et al., 2000; Binder, 1974; Simpson et al., 2009). The findings in this work supported for the observation in a study of Schroder et al. (2011) as milk chocolate containing significantly lower amounts of soluble oxalates compared with those of the darker ones. Whilst some soluble oxalate could be leached to water during cooking and discarded (Savage and Dubois, 2006), soluble oxalate through drinks and confectionary like cocoa products would be all consumed and therefore, could pose a higher risk of hyperoxaluria. Indeed, increases in the urinary oxalate output were found upon 24 h of consumption of dark chocolate (Mendonça et al., 2003; Schroder et al., 2011), but this effect was not observed when milk chocolate was consumed (Mendonça et al., 2003). Similarly, (Charrier et al., 2002) reported that an addition of 25 mL of a non-fat milk (160 mg Ca/100 mL) was sufficient to bind > 80 % soluble oxalate in 245 mL of various commercially sold black tea. Adding low-fat milk to black tea also lowered the urinary oxalate output over 6 h and 24 h, compared to drinking tea only (Savage et al., 2003).

3.2. Effects of of ultrasonic treatments on degradation of soluble oxalate in cocoa powder

In the current study, ultrasonic treatments at 40–80 °C for 1–6 h reduced soluble oxalate in cocoa powder by 5.14–39.96% (Table 4). Ultrasonic degradation of molecules was suggested to result from the high temperatures and pressures released during the collapse stage of cavitation bubbles (Dahlem et al., 1998; Suslick and Nyborg, 1990). In addition, the generation of reactive radical species such as hydroxyl radicals from water molecules (Serpone et al., 1994) could subsequently attack and degrade other compounds in the matrix. Degradation of oxalic acid by sonication was studied in pure chemical with 2–13% reduction being found over 60 min at 40 °C, 40 kHz and 112 W (Dükkanci et al., 2006), but there has been no former study on foods.

The efficiencies of sonication treatments on cocoa soluble oxalate depended on specific combinations of treatment temperatures and time, evidenced by significant interactions between these two factors ($p < 0.001$). Higher temperatures and longer treatment durations generally resulted in more soluble oxalate degraded. Polar organic compounds including oxalic acid could be oxidised by the hydroxyl radicals diffused out from the homolysis of water in cavitation bubbles (Kruus et al., 1997). This oxidation process is usually enhanced by raising treatment temperatures which facilitates the diffusion process and increases collisions between the hydroxyl radicals and the compounds (Entezari et al., 2006). In this trial, the largest reduction of soluble oxalate in cocoa powder (39.96 %) was obtained from the 40 °C + 6 h treatment, which might not be practical for applications due to the long operation time. Alternatively, sonication at 80 °C offered a comparable degradation of 37.75 % cocoa soluble oxalate within a shorter duration (4 h).

Noticeably, while the soluble oxalate contents in cocoa powder sonicated at 40 °C gradually decreased with time, a rise in oxalate concentrations was observed in the samples subjected to 5 h – treatments at 60 °C and 80 °C. These observations suggested the possibility of oxalate formation during the destruction of other substances, as previously recorded in the degradation of longer-chain aliphatic acids and complex compounds. In the sonolytic degradation of butyric acid, oxalic acid was only detected after 5 h of reaction and its concentration went on increasing with time (Dükkanci et al., 2006). Similar note was made by Rehorek et al. (2004) on degradation of five azo dyes. Oxalate was formed after 2–10 h when the dyes concentrations were reduced to 10 % of their initial values, and then steady increased to a maximum level after 20–34 h, following by a linear decrease. Furthermore, ultrasound was also capable in degrading fibres (Wang et al., 2018) which accounted for 17.8 % DM of raw cocoa powder (Valiente et al., 1994). Fibres could naturally bind with oxalic acid and minerals to form fibre – mineral – oxalate complexes (Kelsay and Prather, 1983). Hence, the breakdown of fibres and these complexes, could lead to more soluble oxalic acid released in the sonication medium.

4. Conclusions

Current results suggested that the addition of calcium by its salts or milk products and ultrasonication could be potential methods to reduce oxalate in cocoa powder. The most suitable treatments found were the addition of $CaSO_4.2H_2O$ and high-calcium milk, and sonication at 80 °C for 4 h. However, even when the treatments substantially reduced soluble oxalate contents in cocoa powder, the remaining values were still considerably high. According to The Oxalosis and Hyperoxaluria Foundation (2004), a “low-oxalate diet” recommended for patients at risks of forming kidney stones should contain less than 80 mg of oxalate per day. This amount could be easily made up by approximately 8–10 g of cocoa powders alone. Therefore, the consumption of cocoa powders should be balanced with other oxalate-containing foods, as well as in combination with a diet with higher calcium contents. By using cocoa powder as a model, this study also demonstrated the complexity of calcium-oxalate binding and oxalate degradation in foods and thereby,

Table 3
Solubility of calcium salts in water.

Calcium salt	Solubility (mg/L)	References
Calcium carbonate ($CaCO_3$)	0.017	At 20 °C as stated by the manufacturer Merck, Darmstadt, Germany
Calcium chloride dihydrate (CaCl2. 2H2O)	1280	
Calcium sulfate dihydrate ($CaSO_4$. $2H_2O$)	2.0	
Calcium oxalate ($Ca(COO)_2$)	0.62	At 25 °C by Pedersen (1939)

Table 4
% Reduction of soluble oxalate contents in cocoa powder ultrasonicated at different temperatures and durations (compared to the untreated).

Temperatures (°C)	Durations (h)					
	1	2	3	4	5	6
40	6.79 ± 0.94^{eA}	8.13 ± 0.53^{deC}	9.61 ± 0.21^{dC}	17.90 ± 1.30^{cC}	27.01 ± 0.41^{bA}	39.96 ± 0.48^{aA}
60	6.82 ± 1.21^{dA}	14.06 ± 0.86^{cA}	13.24 ± 1.60^{cB}	20.09 ± 0.40^{bB}	13.52 ± 1.74^{cC}	36.46 ± 0.88^{aB}
80	5.14 ± 0.75^{eA}	10.10 ± 0.86^{dB}	18.80 ± 1.71^{cA}	37.75 ± 1.28^{aA}	23.27 ± 2.05b^{bB}	16.40 ± 1.51^{cC}

Two-way ANOVA analysis

	Between temperatures	Between treatment durations	Interactions of temperatures x treatment durations
F values	5.189	582.4	5.189
	ns	***	***

Mean values within treatment temperatures followed by different lowercase letters are significantly different at $p < 0.001$.
Mean values within the same treatment durations followed by different uppercase letters are significantly different at $p < 0.001$.
Significance: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: non-significant.

suggesting that solutions for reducing soluble oxalate in different commodities would need to be optimized separately.

CRediT authorship contribution statement

Nha. K. Huynh: Methodology, Writing - original draft, Formal analysis, Investigation. **Duyen H.M. Nguyen:** Writing - original draft. **Ha V.H. Nguyen:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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