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Estimated daily intake and risk of prevailing acrylamide content of alkalized roasted cocoa beans

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ABSTRACT

The consistency of the brown color, flavor and dispersability of cocoa solids is the goal of a successful Dutching process of cocoa masse. However, acrylamide, a known probable carcinogen which is one of the by-products of browning reactions, is linked to the strength of alkalis used in the Dutching process. This study used Response Surface D-optimal, 20 experimental runs to determine acrylamide content of beans after using treatment factors; temperature (110–160 °C), time (20–50 min) and K_2CO_3 (10–70% w/v). Subsequently, the alkalized cocoa masses were treated to obtain extracts containing acrylamide using QuEChERS method. The concentrations of the acrylamide were determined using HPLC after which the data was processed by fitting a cubic process order followed by diagnostics to remove outliers. The results yielded optimized process conditions of treatment to yield low acrylamide content, which was later validated to be 7.7×10^{-2} mg/g, at alkali concentration of 29.17% and roasting temperature - time system of 110 °C and 20 min respectively. Though the validated acrylamide content was relatively lower than what has been reported in some European markets, it was found to be still high relative to other markets. This suggest that the application of K_2CO_3 could hold promise of lowering acrylamide concentrations. However, after exposure studies, the validated acrylamide yielded a probable risk of '4 in 1000' adults and '2 in 100' young children consumers of chocolate products, which were highly unacceptable relative to the deminimis ('1 in 1,000,000'). Thus, based on the deminimis risk, further control measures must be sought to produce safer alkalized cocoa masse.

Introduction

The main ingredient for chocolate and other related products are obtained from fermented dried cocoa beans. In many of the chocolate-producing companies, the fermented cocoa beans are deshelled and roasted, broken down to cocoa nibs and ground into a suspension called cocoa masse. Cocoa masse is finally pressed to remove cocoa butter leaving a solid

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cake, which is eventually pulverized into cocoa powder. Along the processing line, the van Houten's process, also called the Dutching process, may be carried out using alkaline salts to treat the processed cocoa beans [1]. A number of advantages have been attributed to the alkalization process. Some of the advantages of the alkalization have been listed as reduction of bitterness, increased dispersability of cocoa powder and the production of consistent brown colors [2]. Acrylamide is often produced in carbohydrate–protein rich systems during roasting above 100 °C. This process leads to the so-called browning reactions also known as Maillard reactions [3]. This reaction decomposes L- asparagine in particular, in the presence of carbonyl compounds that abound in the Maillard process, to produce acrylamide [4]. The thermal processing of cocoa beans making use of relatively high temperatures above 100 °C is a source of worry, since studies have already shown that acrylamide readily forms under such conditions. Several strategies for reducing acrylamide formation have been proposed involving different additives, such as rosemary and amino acids or proteins [5–7]. It has been shown that reduction of pH dramatically reduced the formation of acrylamide during frying and baking of corn chips [8]. The International Agency for Research on Cancer (IARC) has classified acrylamide as a category 2A substance, meaning it is "probably carcinogenic" [9]. This classification has come as a result of intensive studies on the production and hazard identification of foods containing acrylamide.

The presence of acrylamide in foods is a serious problem because two major adverse health effects; carcinogenicity and neurotoxicity have been associated with it [10]. Many studies have suggested a positive association between dietary intake of acrylamide and breast, kidney and endocrine cancers [10–14]. Acrylamide has been reported as targeting neuronal sulphur amino acids and thus, positively associating with neurotoxicity in a dose-dependent manner in test animals [15,16]. There is a report indicating a large per capita consumption of chocolate and its products (up to 8 kg per person per year) [17], meaning consumption of cocoa-based products could be a source of chronic ingestion of acrylamide. However, it is hard to comprehend that some reports indicate that the risks associated with the consumption of the products of cocoa powder is negligible [18]. It is still difficult to understand why at present, there is no enforceable regulatory benchmark for toxicity of acrylamide in foods. Realizing these difficult situations, the Centre for Science in the Public Interest suggested that a limit of acrylamide in fries be set at 77 ppb (7.7×10^{-5} mg/g) as an interim acceptable level [19]. However, this threshold is hardly enforced since it does not have regulatory instrument backing it. The presence of acrylamide in food is termed a "foodborne toxicant", therefore processors are currently grappling with the best way to deal with this type of contamination. It is this challenge that informed EU legislation to accept an ALARA (As Low As Reasonably Achievable) approach to monitor acrylamide in foods [20]. However, as technology improves, it would not be surprising that the ALARA approach would be reviewed for a better and a more sustainable management options. Perhaps, what is more worrying is the lack of consensus of a benchmark and also the approach to be used to assess the risk of the carcinogenicity of acrylamide. In the midst of these challenges, EFSA's scientific opinion on acrylamide continue to give signals of serious health concerns relating to acrylamide [21]. Gradually, consumers are calling for tougher acrylamide control measures that require more stringent benchmarks based on the best, but not the worst processing methods [22]. There is abundant evidence of carcinogenicity of acrylamide [11,13,14,23], but there is also a paucity of information on comprehensive studies on the deminimis risks of acrylamide in chocolate and other products. Thus, the need to harmonize all approaches of risk evaluations and benchmarks in favor of protecting consumers' health rather than presuming the risk due to dietary acrylamide ingestion is negligible cannot be overemphasized. This study which was in two parts, therefore sought to monitor alkalization of cocoa nibs using potassium carbonate, coupled with optimizing the roasting-time cycle of the Dutching process in order to reduce acrylamide formation. Subsequently the deminimis risks of carcinogenicity of the prevailing acrylamide in modeled cocoa products were estimated.

Materials and methods

Materials

Fermented and dried cocoa beans with moisture content ranging between 7% and 9% were obtained from cocoa farmers at Sefwi-Wiawso cocoa-growing areas in Ghana. After bulking to ensure homogeneity, the wastes from the beans were removed by sieving through a 200 mm × 50 mm circular Retsch sieve shaker (AS200 Basic, Germany), operated at 120 rpm. The cleaned cocoa beans samples were then pre-dried in an electric oven (45 °C, 24 h) and later crushed to manually remove the husks. All other chemicals and reagents used in this study were of analytical grade and sourced from Sigma Aldrich, Germany.

Methods

Experimental design

Guided by other studies [24,25], the cocoa nibs were dutched in Response Surface D-optimal design with three independent variables using coded design points presented in Suppl 1. The roasting temperature (A) ranged between 110 and140 °C with, roasting time (B) ranging from 20 to 50 min. A dose range of K_2CO_3 concentrations (C) between 10 and 70% w/v was used as the Dutching agent. The design was deemed satisfactory in terms of the degrees of freedom for the model (9) and residuals (10), with lack-of-fit greater than 3 and pure error greater than 4.

The dutching process

A method which has been described in another study [26] was used for the Dutching process but with slight modification. In the protocol, 500 g of the cocoa nibs were first sprayed with 100 mL solution of K_2CO_3 solutions (10%, 40%, 70% w/v), as the nibs were spread on a 200 mm × 50 mm circular Retsch sieve. The alkali-treated cocoa nibs were then roasted in a conventional Memmert Universal oven (UFE 400, Germany), preheated to the specific temperatures of 110, 125, 132 and 140 °C. The nibs were left to roast for specific times; 20, 27.5, 35 and 50 min. The oven door was only opened to remove the dutched nibs when their specific times were due. After cooling at ambient temperature, each batch of treated cocoa nibs was ground separately in a Moulinex grinder (MC 300161, France) into a fine powder, sealed and kept at room temperature until the next analysis. In order to determine the effect of temperature and time and their interactions on acrylamide levels, a control roasting temperature-time treatment of a batch of cocoa nibs were run under similar conditions but with no alkalization.

Extraction and clean-up of acrylamide in dutched cocoa masses

Extraction of acrylamide was made according to the QuEChERS protocol by accurately weighing dutched cocoa masse samples (2 g), and quantitatively transferring into a labeled 50 mL centrifuge tubes [27]. Hexane (5 mL) was added to the mixture and vortexed (3–5 min). Acetic acid in acetonitrile (1%) and distilled water (10 mL each) were further added, vortexed (3–5 min) and 1500 and 500 mg respectively of $MgSO_4$ and NaCl added. The mixture was vortexed (3–5 min) and subsequently centrifuged (LHW 24,958, Wageningen) at 4500 rpm for 5 min. The resulting acetonitrile phase (1 mL) was siphoned for further analysis.

HPLC determination of acrylamide

The concentration of acrylamide was determined based on the method described, using LC–DAD for quantification [28]. It involved a Cecil-Adept binary pump HPLC coupled with Dynamic Absorbance detector set at 225 nm with Agilent Eclipse Plus C18 column (4.6 mm × 150 mm, 3.5 µm). Column temperature was set at 25 °C and a mobile phase of acetonitrile/water (20:80 v/v) adjusted to pH 3.5 (orthophosphoric acid) was used. Quality control was monitored by spiking 2 g of analytical starch with standardized acrylamide concentrations (20, 50, 100 µg) which gave a mean recovery of 97%, showing sufficient accuracy [29]. The study had a limit of detection and limit of quantification of 0.03 µg/g and 0.1 µg/g respectively and an r^2 of 0.998 for the calibration curve which was used for the study.

Statistical analysis

The data was analyzed by using Design-Expert (Version 7.1, Minneapolis) to fit the summary and study the ANOVA. After evaluating the *p*-value of the model to be significant ($p<0.05$), and also ensuring that lack-of-fit was not significant ($p>0.05$), diagnostics was made to study outliers after which the modeled graphs were obtained for the process variables (A × B, A × C and B × *C*). Optimization was performed with the goal of minimizing the Dutching conditions that will lead to minimized acrylamide content of the alkalized cocoa masse. In order to accelerate the Dutching process, the alkali concentration was targeted at 29.17%, way above the minimum of 10%, but significantly lower than 70% [1]. The output variable (acrylamide content) was then set to the highest importance (5+). The optimized results subsequently yielded Dutching conditions registering a Roasting temperature of 110 °C and a time of 20 min, which were subsequently validated in a separate re-run of the optimized process conditions based on fresh data collected.

Estimated daily intake and risk of acrylamide in chocolate products

The estimated daily intake (EDI) of acrylamide in cocoa was determined (Eq. (1)) using US EPA standard protocols [30]. The validated acrylamide concentration (C_{acryl}) from the dutched cocoa masse obtained (Table 2) was used in the EDI calculation. The remaining elements of the EDI determination were obtained from secondary data. The mass of chocolate products (M_{choco}) consumed per person per year was obtained as 8 kg [17]. Default body weight (BW) of an adult (70 kg) and young children aged 1–3 years (12 kg) were as according to EFSA [31]. The fraction of total cocoa solids (F_{CS}) required in formulating cocoa products were derived based on the definition of chocolate according to Codex Alimentarius (Suppl. 2) [32]. The exposure (EDI) was subsequently calculated using Eq. (1), where the value (F_{CS}) was equal to the statistical distribution of the fraction of total cocoa solids in formulated chocolate products.

$$\mathbf{EDI} = \frac{\mathbf{C_{acryl}} \times \mathbf{M_{choco}} \times \mathbf{F_{cs}}}{\mathbf{BW}} \tag{1}$$

Subsequently, the probable carcinogenicity risk was determined according to Eq. (2) as recommended by US EPA [33] based on a potency factor (PF) of 0.5 $(mg/kg\ bw\text{-}day)^{-1}$. The margin of exposure (MoE) was also calculated based on Eq. (3) as recommended by EFSA [21] using a benchmark dose lower bound 10% ($BMDL_{10}$) value of 0.17 mg/kg bw-day. The risk and margin of exposure were then iterated 100,000 times using @RISK (Palisade, 2018) software.

$$\mathbf{Risk} = \mathbf{PF} \times \mathbf{EDI} \tag{2}$$

$$\mathbf{MoE} = \frac{\mathbf{BMDL_{10}}}{\mathbf{EDI}} \tag{3}$$

Table 1
Treatment conditions for sampled cocoa nibs, control cocoa nibs and their corresponding acrylamide responses.

Run	Input variables[a]			Response variable
	A:	B:	C:	Acrylamide ($\times 10^{-2}$ mg/g)
1	140	50	10	10.84
2	140	20	70	11.14
3	140	50	70	10.41
4	110	50	70	10.07
5	140	20	10	10.43
6	110	20	70	10.08
7	125	20	10	10.22
8	140	35	10	11.42
9	125	35	70	11.14
10	110	50	70	9.96
11	140	20	70	10.40
12	125	50	40	10.83
13	110	50	10	10.34
14	140	50	70	10.52
15	110	35	10	10.56
16	110	20	10	6.73
17	110	20	70	9.93
18	125	35	70	10.18
19	110	35	40	9.85
20	132.5	27.5	40	11.52
21	110	20	0	9.7
22	110	20	0	9.4
23	110	50	0	9.6
24	110	50	0	9.3
25	110	35	0	8.1
26	110	20	0	9.5
27	125	50	0	9.9
28	125	20	0	10.0
29	125	35	0	9.3
30	140	35	0	9.1
31	140	50	0	10.2
32	140	50	0	10.1
33	140	20	0	9.4
34	140	20	0	9.6

[a] Input Variables are; *A*= Roasting temp (°C), *B*= Roasting time (min), *C*= Alkaline concentration (%).

Results and discussion

Table 1 shows the variable acrylamide content of cocoa masse obtained from the treatments with and without alkalization. Cocoa masse with alkalization had acrylamide content ranging from 96.73×10^{-2} mg/g to 11.52×10^{-2} mg/g (Table 1) and the one without alkalization ranging from 8.1×10^{-2} mg/g to 10.2×10^{-2} mg/g (Table 1).

Analysis of variance

The effect of each of the impacts of the variables (A, B and C) on the acrylamide content of the dutched cocoa nibs as studied is presented in the ANOVA (Suppl 3). The relationship was presented as a mathematical model of the variables independently and interactively, between the process factors and the response factor (acrylamide, y), fitting a second order regression (Eq. (4)).

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j + \sum_{i=1}^{1} \sum_{j=1}^{1} \sum_{z=1}^{1} \beta_{ijz} x_i x_j x_z + \sum_{i=1}^{1} \sum_{z=1}^{1} \beta_{iz} x_i^2 x_z + \sum_{i=1}^{1} \sum_{z=1}^{1} \beta_{iz} x_i x_z^2 \quad (4)$$

where: y = dependent variables (acrylamide concentration), β_0 is the value of the fixed response; β_i and β_{ii} are respectively linear and quadratic coefficient of the independent variables, β_{ij} and β_{ijz} is interaction coefficient and x_i, x_j, x_z, are the processing factors, where $i = A$, $j = B$ and $z = C$,

This regression appropriately represents the behavior of the system, since the coefficient of determinations; r^2 (92.83%) and adjusted r^2 (80.54%), indicated that the model is significantly responsible for the response under study. Thus, the model obtained was significant ($p<0.05$) and can be used to navigate responses in the data set or the design space of the study (Suppl 3). Similarly, a review of the Dutching process variables indicated that though the roasting time (B) was not significant ($p>0.05$), roasting temperature (A) and alkali concentration (B) were significant ($p<0.05$) (Suppl. 3).

Table 2
Prediction and validation of acrylamide concentration of cocoa masse based on the optimization under alkalized and non-alkalized conditions.

Input variables			Validated response variables ($\times 10^{-2}$ mg/g)			
Roasting time (min)	Roasting temperature (°C)	Alkali concentration (%)	Prediction	95% CI low	95% CI high	Validated value
20	110	29.17	6.7	5.2	8.91	7.7
36	110	0	8.5	8.1	8.83	8.7

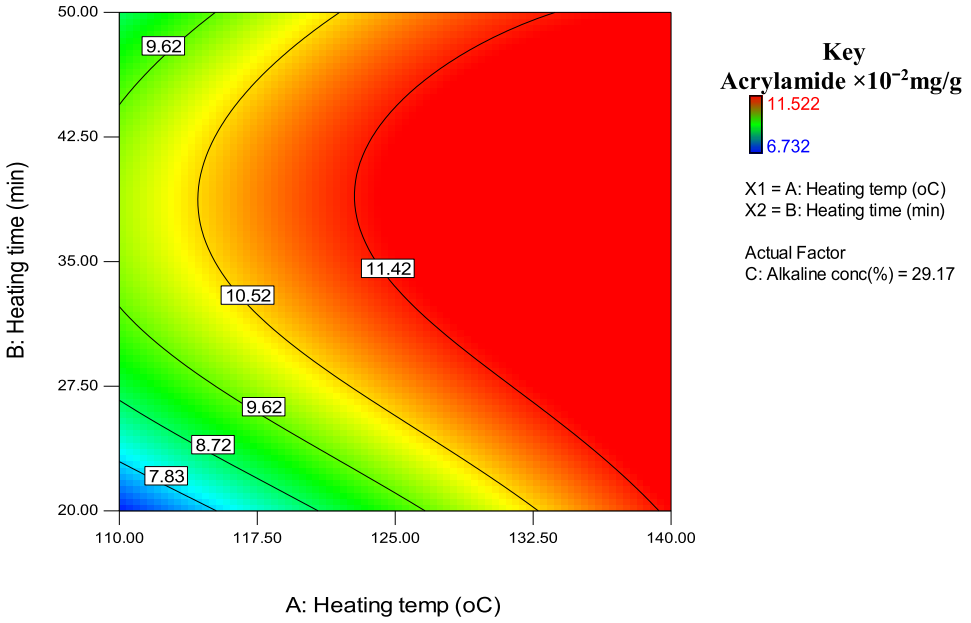

Fig. 1. Acrylamide ($\times 10^{-2}$ mg/g) response in alkalized cocoa masse treated within alkaline concentration of 29.17% and roasted between 110 °C and 140 °C within 20 and 50 min.

The results further showed that the interactions; A × B, A × C, and B × C were equally statistically significant ($p<0.05$). This implies, the roasting time (A) and alkali concentration (C) were both independently, and also acting together (A × B, A × C and B × C), directly influenced the formation of acrylamide in the cocoa masse. Similarly, all the three process factors (A × B × C), acting together, significantly ($p<0.05$) influenced the content of acrylamide in the cocoa masse. Thus, it did not matter that the time of processing, acting alone, did not have any significant ($p>0.05$) impact on the formation of acrylamide. On the other hand, the model representing treatment without alkalization of the cocoa nibs (Suppl. 4), presented a model that was significant ($p<0.05$), with only roasting temperature ($p<0.05$) impacting on acrylamide content but not roasting time ($p>0.05$).

The optimized and validated process conditions produced acrylamide levels of 7.7×10^{-2} mg/g in cocoa masse as presented for the alkalized treatment in Table 2 relative to 8.7×10^{-2} mg/g which was obtained without alkalization (Table 2). These two values are significant since they fall within the range of 95% low and high confidence intervals. Thus, based on the report that acrylamide formation in cocoa-based products ranges between 0.002 mg/g and 0.826 mg/g [34], the validated values of acrylamide obtained from the studies, with alkalization (Table 2) or without alkalization (Table 2), are consistent with such an observation.

Effect of input variables

From Fig. 1, it is seen that within the limits of the temperatures and times of processing, roasting temperature coupled with roasting time progressively increased the production of acrylamide at a fixed alkaline concentration of 29.17%. This observation supports the role of temperature and time of roasting on the acrylamide content food products, which have been documented as key agents of the kinetics of the browning process [35]. Thus, the increase in acrylamide formation with increasing brown color which is associated with roasting was expected.

Indeed, several reviews have suggested possible models that link roasting temperature and time to Maillard browning products and how these browning products are positively related to acrylamide formation [6,36,37]. However, the dynamics during roasting without alkalization, as presented in Suppl 5, was different. The ANOVA (Suppl 4) clearly shows that roasting time was not significant and this fact was perhaps responsible for the non-progressive increase in acrylamide for the non-alkalized samples. This observation was unexpected because, it is generally accepted that roasting time impacts

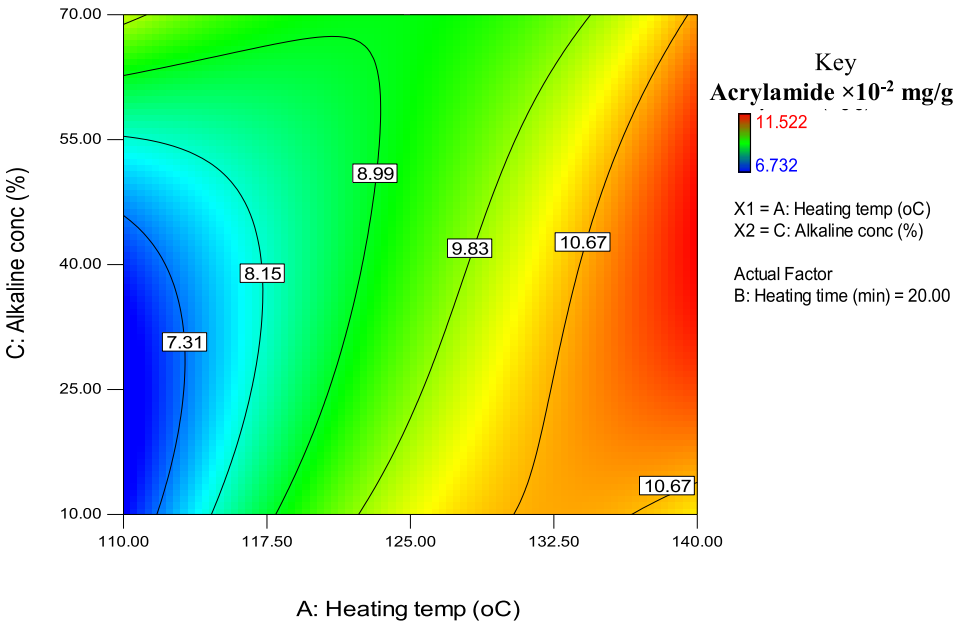

Fig. 2. Acrylamide ($\times 10^{-2}$ mg/g) response in alkalized cocoa masses treated within alkaline concentrations ranging between 10% and 70% and roasted for 20 min between temperatures of 110 °C and 140 °C.

on acrylamide content positively as reported in other studies. Again, it was expected that the alkalized cocoa masse would rather produce greater quantities of acrylamide due to the higher alkaline conditions believed to drive the formation of intermediates of the Maillard reaction [38,39]. However, the minimum acrylamide concentration obtained in the treatment without alkalization (8.7×10^{-2} mg/g), was relatively greater than the acrylamide content of treatment with alkalization (7.7×10^{-2} mg/g). This observation may be related to the complex nature of inhibition of acrylamide formation cited from sources such as food antioxidants [40] and varying salts of carbonates [7,41] that are believed to alter the course the formation of the intermediates responsible for the Maillard reaction products. The influence of these factors suggest that though roasting and time variables have direct impact on acrylamide formation in such foods as fried potatoes and fried rice [42], in alkalized cocoa masse, these variables might have indirect impact routing through alkaline sensitive pathways. The impact of alkali concentration and roasting time on the production of acrylamide is presented in Fig. 2. In this case, at a roasting time of 20 min coupled with low roasting temperature (110–120 °C), and a wider range of alkali concentrations of up to 60%, still maintained acrylamide concentrations of up to 8.99×10^{-2} mg/g.

Again, the effect of alkali concentration and roasting time at a low temperature of 110 °C presented the dynamics of acrylamide as shown in Fig. 3. It is seen that low acrylamide concentrations of up to 9.24×10^{-2} mg/g could be obtained when roasting time was less than 30 min, even with a wider range of over 55% alkali concentration.

Though low acrylamide concentration formed within a wider tolerable range of alkali concentrations (Figs. 2 and 3), temperature and time of processing were key in the formation of acrylamide. This is so because the interactions between these three input variables ($A \times B \times C$) (Suppl 3), obviously influenced the production of acrylamide in the cocoa masse.

Precisely how alkalinity impacts on the formation of the pool of carbon fragments formed in the intermediate stages of the Maillard process from which acrylamide formation occurs, is not clear. However, from the mechanistic point of view, acidic environments might lead to the protonation of the nitrogen in the amino group (NH_2) of asparagine, a key precursor of acrylamide [3]. Such protonation leads to the formation of strong electrophiles ($^{(+)}NH_3$) that might have little affinity for the already partially positively charged (δ^+) carbonyl carbon of the sugar. Since the formation of the glycosylamine subsequently yields Maillard reaction products, any variable that disrupts this reaction would certainly prevent browning [3]. On the other hand, under strong alkaline condition, the free amino group can be deprotonated into a nucleophile ($-NH^-$) that would readily attack the carbonyl carbon to initiate glycosylamine formation. Such alkaline environment would subsequently speed up Maillard reaction leading to the formation of brown products along which acrylamide may be formed [39]. From an industrial viewpoint, the Dutching process is required to produce consistent brown color and flavor that is required in cocoa products for their aesthetic appeals [2]. For consistency of brown color and subsequent low acrylamide formation, the events ranging from weak to strong alkaline environments can be exploited to deliver stable brown color while controlling acrylamide formation. Thus, any base that could offer such an alkaline environment with concomitant low acrylamide levels would certainly be advantageous.

At high temperature (132.50 °C) (Figs. 1 and 2) and a lower alkali concentration (<25%), persistently high acrylamide content (10.67 mg/g) was produced. Thus, it is recommended that processors avoid high processing temperatures at all cost.

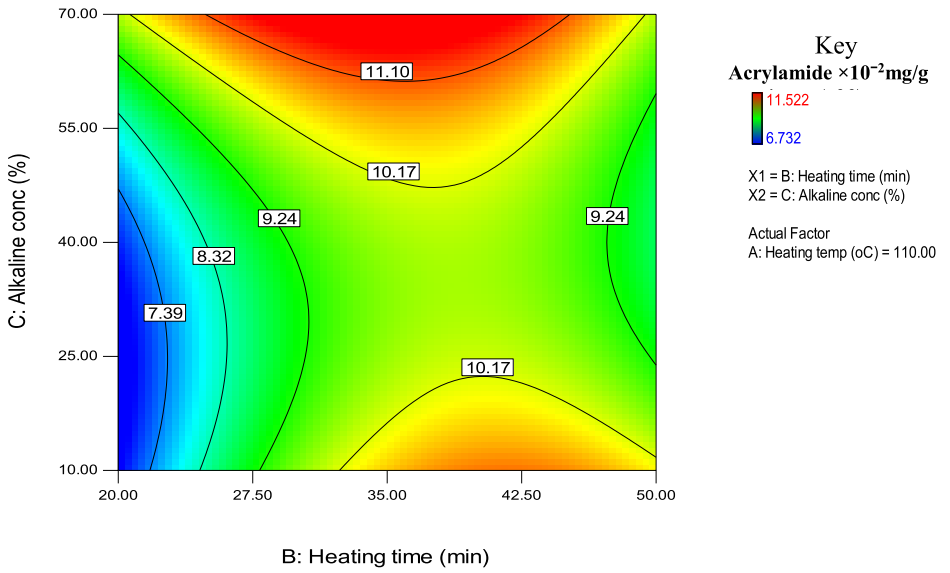

Fig. 3. Acrylamide ($\times 10^{-2}$ mg/g) response in alkalized cocoa masses treated within alkaline concentrations ranging between 10% and 70% and roasted at 110 °C between 20 and 50 min.

Indeed, only Roasting time, as an input variable, though not a significant ($p<0.05$) factor on acrylamide formation, can still be a valuable tool to manipulate within alkali concentrations of up to 55%. It is above this concentration that the acrylamide content builds up to above 10 mg/g, even at a low roasting temperature of 110 °C. The observation is backed by the ANOVA (Suppl 3) that shows significant ($p<0.05$) interactions between the concentration of alkalis and roasting time (B × C), acting together. The role of alkalinity of a base in acrylamide formation might be more complex than suggested in this study, but the results clearly show that a wider range of alkali concentration (10–55%) corresponded to consistently low acrylamide concentration (8.15×10^{-2} mg/g) (Fig. 3). Acrylamide concentration only gradually increased as temperature increased above 120 °C. The results also suggest that the nature of the alkali used is key, and that when the alkalinity of the base increases, it might lead to greater acrylamide formation even at a low processing temperature of 110 °C.

A recent survey of cocoa products in German retail shops, concluded that the range of acrylamide concentrations was between less than 3.9×10^{-5} mg/g and up to 4.9×10^{-4} mg/g [18]. Also a report of acrylamide concentrations ranging between 9×10^{-6} mg/g and 1.747×10^{-3} mg/g has been published in other studies [43]. Relatively, the validated level of acrylamide obtained in this current study (7.7×10^{-2} mg/g) is higher compared to samples collected from German retail shops. Studies have also indicated a relatively higher acrylamide content ranging between 1.5 mg/g and 15.6 mg/g [24]. Clearly, these reported values of acrylamide are higher than what was obtained in this present study (7.7×10^{-2} mg/g). By contrast, the validated results obtained in the current study appears to be much lower relative to what has been reported in Scandinavian countries and also in the UK and US [44].

EDI and probable carcinogenic risk

The validated acrylamide content of the cocoa masse in this study (7.7×10^{-2} mg/g) (Table 2) was relatively higher than what was reported in a study of cocoa products on German markets (3.9×10^{-5}–4.9×10^{-4} mg/g) [18]. They concluded in their study that there was low perceived risk as benchmarked on the general German signal value of 1000 µg/kg. Other studies have also reported relatively higher acrylamide content ranging between 170 mg/g and 230 mg/g [10]. Though zero risk is not a realistic goal, these reported high levels of acrylamide must not be taken lightly because the presence of carcinogens in food, to a large extent is a cause for concern [14]. From Suppl. 6 and 7, all the central tendency measures of cocoa products with and without alkalization among adults seem to be lower, relative to the threshold exposure of the general population (0.3–0.8 mg/kg bw-day) [44]. Comparatively, young children were seen to be exposed at even higher levels at all the central tendency measures. A study in Jeddah, Saudi Arabia, on dietary exposures to acrylamide from foods containing chocolate, shows similar results where the exposures decreased significantly as the age of the consumers increased [45].

A casual look at the EDI suggests a safe situation according to the benchmark suggested in some studies [18]. However, the risk profile as presented in Table 3 shows a different situation altogether. For the adult population, a modal risk of 0.0042 (4 out of thousand consumers), is significantly higher relative to the acceptable deminimis (10^{-6} or 1 in million consumers). It also shows that young children are even at a greater risk of 0.02 (2 out 100) relative to the deminimis. For young children, it is their lower body weights that puts them at risk [46]. One sure way of drawing further attention to the problem is to reduce the presence of the hazard in the food product. Thus, this current results contradicts the findings that acrylamide levels in cocoa and chocolate products on the German markets is safe. The discrepancy might be the measure

Table 3
The metrics of the risk based on the alkalized and non-alkalized cocoa masse validated acrylamide risks projected in varieties of cocoa products for averaged weight adult and young children consumers.

		Min	Max	Mean	Mode	Median	5th	95th
Risk[a]	Adult	0.0022	0.005	0.0036	0.0042	0.0036	0.0024	0.0049
	Young children	0.013	0.029	0.021	0.022	0.021	0.014	0.028
Risk[n]	Adult	0.0025	0.0056	0.0041	0.0035	0.0041	0.0027	0.0055
	Young children	0.015	0.033	0.024	0.031	0.024	0.016	0.032
MoE[a]	Adult	17.0	38.0	25.0	17.0	24.0	18.0	36.0
	Young children	2.91	6.51	4.24	2.93	4.03	3.00	6.13
MoE[n]	Adult	15.0	34.0	22.0	15.0	21.0	16.0	32.0
	Young children	2.58	5.76	3.75	2.56	3.57	2.66	5.43

[a] alkalized cocoa masse; na: non- alkalized cocoa masse.

of the risk profile used in the other studies. In their study, the approach centered on a hazard-based threshold method, requiring only a reference dose. However, this current risk study was based on the potency factor approach, requiring comparison to deminimis (10^{-6}). Certainly, a modal risk of '4 out of 1000 consumers' of the adult population and '2 out of 100' of the young children chocolate products consumers, is a situation that must not be taken lightly. Similarly, a margin of exposure (MoE) value of 17 for the adult population and 4 for the young children population, again, buttresses the high risk consumers are exposed to since the threshold of acrylamide has been fixed at 10,000 [47]. Relative to what prevails in cafeteria foods in Jeddah, the MoE values obtained in this current study is lower. However, it must be noted that the higher the value of the MoE, the less likely it is for the concentration of the acrylamide ingested from foods to reach toxic levels. Thus, since a MoE threshold of 10,000 and above, indicates safe levels of acrylamide exposure, the findings of the present study (Table 3) imply both the adult and young children consumers of chocolate and their products are at risk.

Conclusion

In spite of the fact that the study optimized the Dutching condition to obtain a comparatively low acrylamide content of the cocoa masse, an acrylamide content of 7.7×10^{-2} mg/g was achieved. This acrylamide content yielded modal exposures of respectively 0.008 mg/kg bw-day and 0.039 mg/kg bw-day for adults and young children consumers. Subsequently, the modal exposures in alkalized cocoa masse yielded carcinogenic risks of '4 in 1000' adults and '2 in 100' young children consumers of cocoa-based chocolates. An extremely low modal MoE values of 17 (<10,000) and 3 (<10,000) for adult and young children consumers respectively are unacceptably so high and also support the trend of the high carcinogenic risks reported. The study therefore indicates that the use of risk-based approaches in evaluating risk may be the best approach, otherwise safety of acrylamide content in foods may lead to erroneous judgments and compromise. It is suggested that in order to appreciate the extent of the carcinogenic risks of acrylamide in chocolate and other cocoa products, and sustain public health concern, the approach of assessing such risk must rather be based on the deminimis risk benchmark rather than lazed reference dose.

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Declaration of Competing Interest

The authors declare they have no actual or potential competing financial interests.

CRediT authorship contribution statement

Isaac W. Ofosu: Conceptualization, Formal analysis, Resources, Supervision, Validation, Writing - review & editing. **Gloria M. Ankar-Brewoo:** Investigation, Validation. **Herman E. Lutterodt:** Investigation, Validation. **Edmund O. Benefo:** Writing - original draft. **Celestina A. Menyah:** Data curation.

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Supplementary material

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