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Influence of Cookies Formulation on the Formation of Acrylamide

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Abstract This study examined the effects of cookie ingredients and cookie formulation without adding leavening agents, sodium bicarbonate and ammonium bicarbonate, sucrose, glucose, fructose and adding chitosan on the pH, water activity, browning index, reducing sugar of cookies and the formation of acrylamide. High browning index value (218) of control cookies was found, as compared to a combination of ingredients, model cookies (33), cake flour and water during 15 min baking. Higher browning index value (83) of model cookies with the addition of shortening did not show a high acrylamide concentration. The highest mitigation (55.2%) of acrylamide formation was obtained by removing ammonium bicarbonate in control cookie formulation. The formation of acrylamide showed a positive correlation with the cookie baking time. The baking time significantly influences the physicochemical properties of cookies. Ammonium bicarbonate was the most effective ingredient in terms of causing the formation of acrylamide in cookie formulation. The addition of chitosan was also able to mitigate the formation of acrylamide during baking.

Keywords: *acrylamide, cookie, reducing sugar, sodium bicarbonate, ammonium bicarbonate*

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1. Introduction

To understand the interactions that occur in a cookie formulation with reducing sugar and shortening, we used a model cookie recipe with no added shortening, skim milk, sodium chlorine, high-fructose corn syrup and ammonium bicarbonate to study the role of calcium salts and reducing sugars in the formation of acrylamide. Making the model cookies with no shortening or with fortified calcium salts as supplements and reducing sugars associated with the formation of acrylamide allowed for the effect on the cookies to be thoroughly studied. In this study, different types of calcium salts as calcium supplements for reducing the formation of acrylamide in the model cookies without shortening and their relation to the reducing sugar content were also investigated. The quality characteristics of the model cookies and the cookies fortified with calcium salts were also evaluated to develop better strategies to reduce the acrylamide content of baked products.

Cookie types and ingredients vary greatly, so that physical and chemical changes may occur simultaneously. Cookie dough undergoes changes in appearance, structure and taste during baking. The caramelization and Maillard reaction taking place are responsible for the resultant desirable color, cookie taste and even the potential cancer-causing chemical, acrylamide [1]. Acrylamide is a potential neurotoxin genotoxin compound which occurs in thermal-processed carbohydrate-rich food products [2]. It has been demonstrated that acrylamide can be generated by oils and nitrogen-containing compounds in food heated above 100°C. High levels of acrylamide (150-4000 ng/g) can be found in fried and carbohydrate rich baked foods [3]. However, no acrylamide has been detected in unheated or boiled foods [3]. In general, aminal derived heat-treated foods such as poultry, meat and fish, contain lower amounts of acrylamide compared to carbohydrate-rich foods, such as potato chips, bread, bakery wares, coffee and crisps at an elevated temperature [3]. The fatty acid oxidation product, acrolein (CH_2=CH-CHO), can react with ammonia to form CH_2=CH-CHOH(NH_2), which could produce acrylamide by reacting with asparagine or by oxidation to form an N-glycoside, followed by generating acrylamide via oxidation [4]. A mechanism for the formation of acrylamide from the reaction of asparagine and a carbonyl-containing compound in fried mid oleic sunflower oil (205°C) has been demonstrated by Zyzak et al. [5]. Food processing at elevated temperature has been reported as the main source of acrylamide for food products [6]. Rearrangement of amino acids in heat-processed foods could also generate acrylamide [7]. However, the essential pathway of acrylamide formation is still related to the Maillard reaction [8,9]. Seeking more methods to mitigate acrylamide formation in bakery products is still an important issue for the snack and baking industry.

The Schiff base formation is proposed as the first reaction step for acryalmide generation. Jung et al. [10] demonstrated that the conversion of free nonprotonated amine to nonnucleophilic protonated amine could be enhanced by lowering the pH of a food system. It has been clearly established that lowering the pH of potato chips and cookies could effectively mitigate acrylamide formation;

adding an acidulant to lower the pH values of French fries and corn chips to mitigate the formation of acrylamide has been well elucidated [10].

Mitigating strategies to reduce the formation of acrylamide in heated foods have been proposed in last decade, including adding calcium salts [11,12], using sucrose instead of reducing sugars such as fructose and glucose [13,14,15], decreasing the free asparagine amount in heated food systems by asparaginase [16,17], diluting the asparagine concentration with glycine addition [18] and replacing ammonium containing agents with baking powder [19].

Chitosan is the cationic (1-4)-2-deoxy-<beta>-D-glucan, which is a deacetylated derivative of chitin from the exoskeletons of crustacean shellfish, such as shrimp. Chitosan-containing amino groups can react with the carbonyl group of reducing sugar to generate Maillard reaction products [20]. Therefore, the possibility of using ammoniated polymers, chitosan for blocking the carbonyl groups of neutral reducing sugars will be evaluated in this research. Evaluating the acrylamide formation during the baking process and its amount relating to various individual ingredients could help the food industry develop more effective methods to mitigate the formation of acrylamide in bakery products. Leavening agents such as ammonium bicarbonate enhance the formation of acrylamide [15]. Moreover, sodium bicarbonate as a chemical leavening mitigates the formation of acrylamide by over 60%. Amrein et al. [15] propose that asparagine is also a main factor for acrylamide formation.

To confirm the interactions of nonfat dry milk, sodium chlorine, high-fructose corn syrup, sugar and shortening in cookie formulation, a model cookie recipe without sodium bicarbonate or ammonium bicarbonate addition was examined to study the role of leavening agents in the formation of acrylamide. Making the cookies with chitosan associated with the mitigation of acrylamide allowed for the effect on the cookies to be thoroughly studied. Therefore, the aim of this study was also to investigate particular ingredients regarding the formation of acrylamide in cookies and model cookies with flour and water. Physicochemical characteristics of the cookies were also evaluated to develop better strategies to reduce the acrylamide content of baked products.

2. Materials and Methods

2.1. Raw Materials and Chemicals

Cake flour used in this study was purchased from the Cha Hwa Corporation (Taichung, Taiwan). Skim milk, sucrose, shortening, 75% high fructose corn syrup, sodium chloride, food grade sodium bicarbonate and ammonium bicarbonate were obtained from a local store (Fu Shen baking ingredient store, Keelung, Taiwan). 50-190 kDa low molecular weight chitosan with deacetylation degrees greater than 75% was purchased from Sigma-Aldrich (St. Louis, MO, USA).

$^{13}C_3$-lableed acrylamide and D-glucose were supplied by Sigma Aldrich (St. Louis, Missouri, USA). Potassium sodium tartrate, 3,5-dinitrosalicylic acid, potassium sodium tartrate, sodium hydroxide, sulfuric acid were obtained from Panreac (Barcelona, Spain). All chemicals used in this work were of analytical grade. Acrylamide 99.9% was obtained from J.T. Baker (Phillipsburg, NJ, USA). Oasis HLB (6 ml, 200 mg) and Oasis MCX (3 ml, 60 mg) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA, USA).

2.2. Cookie Preparation and Physicochemical Properties of Cookies

The cookie recipe followed the American Association of Cereal Chemists (AACC) method 10-54 (80.0 g cake flour, 17.6 g deionized water, 34.8 g sucrose, 0.8 g non-fat dry milk, 1.0 g sodium chloride, 0.8 g sodium bicarbonate, 32.0 g all-purpose shortening, 1.2 g high fructose corn syrup and 0.4 g ammonium bicarbonate) (AACC, 2000). Model cookie recipes were modified by the addition of 80 g cake flour, 17.6 g deionized water with each individual cookie’s ingredients as shown in Table 1. The model cookie base recipe: cake flour and water, with the addition of particular ingredients (sucrose, fructose, glucose, shortening, sodium bicarbonate, ammonium bicarbonate, salt, skim milk), was evaluated. Chitosan (1% of low molecular weight chitosan in cake flour weight basis) was added to the cookie formula. The recipe for the cookies without the addition of leavening agents was modified from the AACC method 10-54 (AACC, 2000). The cookie dough was rolled out to a thickness of 2 mm and cut with a circular cookie cutter (diameter: 60 mm). The cookie dough was baked in an electric oven (Chung Pu Baking Machinery Co., LTD, Taichung, Taiwan) at 205°C for 10, 15 and 20 min.

The thickness and diameter of cookie samples after baking and cooling down were based on the average of ten measurements by using a Venier Caliper. The cookie spread ratio was obtained from dividing the diameter by the thickness of the cookie. The expansion ratio was calculated as (volume of model cookie)*100%/(volume of cookie) by a sucrose granule displacement method. The effect of ingredients on the pH of the cookies and model cookies was measured by the method of Hwang et al. (2008), using a pH meter (pH meter, Oakion pH/Ion 510, Singapore). The water activity of the cookie was determined using a Thermoconstanter Novasina RTD 33 TH-1 avumeter (Novasina Co. Ltd., Pfaffikon, Switzerland).

2.3. Determination of Acrylamide

Cookies were blended with a pulverizer (D3V-10, Yu Chi Machinery Co., Ltd., Chang Hua, Taiwan). A 2 g cookie sample was weighed and transferred into a 50 ml centrifuge tube; 15 ml of deionized water was added and mixed in a 50°C reciprocal shaker bath for 60 min. The mixture was centrifuged at 5°C for 20 min at 9000 rpm. The supernatant was filtered through a nylon filter (0.45 μm). The HLB/MCX cartridge was conditioned with 5 ml and 3 ml methanol followed by 5 ml and 3 ml of deionized water, respectively. The filtrate (1.5 ml) was then passed through an Oasis HLB/MCX cartridge to absorb the acrylamide, and the filtrate was discarded. The cartridge was then washed with 3.5 ml double distilled water and the first 0.5 ml filtrate was discarded, while the remaining 3.0 ml eluent was collected in a brown glass tube. The eluent was concentrated under vacuum before HPLC analysis.

Table 1. Cookies ingredient specification

Formula	Ingredient specifications	Symbol
1	Flour 80g	Flour
2	Flour 80g, water 17.6g	Flour+ water (a)*
3	Flour 80g, water 17.6g, sucrose 34.8g	a + sucrose
4	Flour 80g, water 6g, fructose 46.4g	a + fructose
5	Flour 80g, water 17.6g, glucose 34.8g	a + glucose
6	Flour 80g, water 17.6g, NaCl 1g	a + NaCl
7	Flour 80g, water 17.6g, nonfat dry milk 0.8g	a + milk
8	Flour 80g, water 17.6g, shortening 32g	a + shortening
9	Flour 80g, water 17.6g, $NaHCO_3$ 0.8g	a + $NaHCO_3$
10	Flour 80g, water 17.6g, NH_4HCO_3 0.4g	a + NH_4HCO_3
11	Flour 80g, water 17.6g, sucrose 34.8g, shortening 32g, $NaHCO_3$ 0.8g, high gructose corn syrup 1.2g, NH_4HCO_3 0.4g, NaCl 1g, nonfat dry milk 0.8g	AACC** (C)
12	AACC, chitosan 0.8g (1%)	C + chitosan
13	AACC formula without $NaHCO_3$ addition	C - $NaHCO_3$
14	AACC formula without NH_4HCO_3 addition	C - NH_4HCO_3
15	AACC formula without $NaHCO_3$ and NH_4HCO_3	C - $NaHCO_3$-NH_4HCO_3

* a: Flour+ water
** AACC formula.

Acrylamide concentration of the cookies and model cookies were evaluated using the HPLC method [8]. The HPLC system (D2000) consisted of L-2300 column oven, an L-2130 pump, L-2400 detector and L-2200 autosampler (Merck, Hitachi, Kent, UK). Chromatographic separation was accomplished by a Capcell Pak C_{18} AQ S5 column (5 µm, 4.6 mm × 250 mm) (Shiseido, Tokyo, Japan) using deionized water at a flow rate of 0.7 ml/min at 25°C. Injection volume: 20 µl and the mobile phase was 100% double distilled water. The 400 µl sample was spiked with 100 µl of $^{13}C_3$-labeled acrylamide along with 0.5 mL of double distilled water. The acrylamide eluate (2µl) of HPLC was injected into a Mass Spectrometer (QTRAP 5500, ABSciex, USA). Mobile Phase: (a) 0.1% (v/v) formic acid/water; (b) 0.1% (v/v) formic acid/methanol. Source temperature: 600°C; electrospray capillary voltage of 5.5 KV; collision energy of 30V. Data acquisition was performed in the selected ion monitoring mode. The ions monitored for identification of acrylamide in the sample were m/z 72.04 for acrylamide and m/z 75.04 for $^{13}C_3$-labeled acrylamide. Full scan analyses were performed in the mass range 50-200. The acrylamide calibration curve was in the range of 5.208-500 ng/g.

2.4. Determination of Reducing Sugars and Amino Acid Analysis

The cookie samples was ground and 0.1 g was taken and added to 10 ml of 1.5 M sulfuric acid and heated for 20 min in a boiling water bath. 12 ml of 10% sodium hydroxide was added when the solution was cool. The solution was filtrated with Whatman Grade No. 1 filter paper and made up to 100 ml with double distilled water. Standard glucose solution was used in the assay for reducing sugar. The quantitative determination of reducing sugars was measured by a dinitrosalicylic acid reducing sugar assay following the method of Ilyina et al. [23].

2.5. Determination of Cookie Browning Index

The color of the cookie was determined with a spectrocolorimeter (TC-1800 MK II, Tokyo, Japan) using the L* (lightness), a* (redness/ greenness) and b* (yellowness/blueness) color scale. The white tile and black cup were conducted to standardize the spectrocolorimeter. Cookie samples were ground and loaded onto a quartz sample cup with three measurements for each sample, and triplicate determinations were recorded per treatment [24]. The brown index (BI) was determined using the following equation:

$$BI = 100 * [x - 0.31] / 0.17$$

where $x=(a^*_{sample} + 1.75 L^*_{sample})/(5.645L^*_{sample} + a^*_{sample} - 3.012b^*_{sample})$.

2.6. Statistical Analysis

A completely randomized block design was used with three replications per treatment. Data were analyzed by analysis of variance using the SPSS statistic program (SPSS version 12, 1998). Duncan's multiple range test was used to identify the difference between treatments at a 5% significance level ($p<0.05$).

3. Results and Discussion

3.1. pH of Cookies and Model Cookies

The pH of the cookies and model cookies decreased with increasing baking time (Table 2). This may have been because the amino group reacted with a carbonyl source in the cookie system under heat from Schiff's base during the Maillard reaction, which results in the elimination of water [25]. Hydrogen ions might be released by the Amadori rearrangement and generated melanoidin, which decreased the pH of the cookies and model cookies. The addition of particular ingredients into the model cookies of cake flour and water did not decrease the pH more than 2 units from model cookies during 20 min of baking (Table 2). The pH of AACC cookies dramatically decreased from pH 9.1 to pH 6.2 (Table 2). Sodium bicarbonate gradually decomposes into carbon dioxide, sodium carbonate and water. Sodium carbonate has a slightly alkaline taste. Ammonium bicarbonate dissolves in water to give a mildly alkaline solution and decomposes above about 36°C into carbon dioxide, ammonia, and water. The addition of chitosan into the cookie formulation did not change the pH of the cookies. The cookies' pH increased slightly after the addition of chitosan perhaps because the chitosan did not dissolve and react with other ingredients during baking (Table 2).

Clawson and Taylor [26] reported that the pH decreased from the initial pH values of 6.12 and 7.81 to 5.88 and 6.32, respectively, when ground wheat samples were cooked in sealed glass ampoules for 70 min at 121°C. Hamlet and Sadd [27] also showed that the pH dropped from 5.91 to 4.73 in a pressure-cooking apparatus at 180°C for 20 min using a basic flour, water and salt dough model system. Reductions in pH were attributed to the Maillard reaction [28] and caramelization of sugar (pH 3-4). The pH of all cookies decreased after baking, in comparison to baking cookies for 10 min (Table 2). In general, the model cookies without sodium bicarbonate addition had a lower pH (pH 7.00) after 10 min baking time.

The Aw of the cookies and the model cookies were between 0.49 and 0.67 after 10 min baking time. When the Aw was between 0.7 and 0.5, hexose formed hydroxymethylfurfural in the cookie system at the middle stage of the Maillard reaction [29]. The model cookie recipe of cake flour and water with sucrose or glucose groups had higher Aw values (Aw = 0.67) than other groups, indicating that the sugars prevent water from evaporating during the first 10 min baking time. The model cookie recipe of cake flour and water with fructose had the lowest Aw value (0.55) among the glucose and sucrose groups; this may be due to the fructose making it easier to react with amino acids at the initial stage of the Maillard reaction for dehydration [25]. Katz and Labuza [30] proposed that cookies would be crispier when the Aw is below 0.39. However, the Aw of the model cookie with fructose remained at a higher value (Aw = 0.3) after 15 min baking compared to the glucose and sucrose groups (Aw = 0.15 and 0.21, respectively), indicating that fructose will bind with water better than other groups do for further baking. The water activity of cookies and cookies without the addition of leavening agents was between 0.48 and 0.54, respectively, after 10 min baking. It indicates that leavening agents will not significantly decrease the water activities of cookies during baking. However, these cookies were not crispy enough. There were no differences among all water activities of the model cookies, and they were below 0.3 after 15 min baking.

Table 2. pH of cookie and model cookies baked for 20 min

	pH		
	10 min	15 min	20 min
Flour	5.90 ± 0.03 ^{Cb}	5.75 ± 0.16 ^{Bb}	5.07 ± 0.03 ^{Ba}
Flour+water (a)	5.73 ± 0.09 ^{BCb}	5.93 ± 0.13 ^{BCDb}	5.38 ± 0.18 ^{Ca}
a + sucrose	5.85 ± 0.20 ^{Cb}	5.99 ± 0.15 ^{CDb}	5.04 ± 0.16 ^{Ba}
a + fructose	5.49 ± 0.06 ^{Ac}	5.15 ± 0.06 ^{Ab}	4.91 ± 0.07 ^{Ba}
a + glucose	5.63 ± 0.10 ^{ABc}	5.17 ± 0.09 ^{Ab}	4.13 ± 0.09 ^{Aa}
a + NaCl	5.88 ± 0.04 ^{Cb}	5.82 ± 0.02 ^{BCb}	5.49 ± 0.06 ^{CDa}
a + milk	5.93 ± 0.09 ^{Cb}	6.11 ± 0.06 ^{Dc}	5.46 ± 0.07 ^{CDa}
a + shortening	5.82 ± 0.07 ^{Ca}	6.10 ± 0.06 ^{Db}	5.72 ± 0.14 ^{Da}
a + $NaHCO_3$	10.33 ± 0.08 ^{Fc}	9.61 ± 0.04 ^{Gb}	8.54 ± 0.46 ^{Fa}
a + NH_4HCO_3	6.65 ± 0.10 ^{Da}	6.44 ± 0.15 ^{Ea}	5.77 ± 0.03 ^{Db}
AACC (C)	9.13 ± 0.17 ^{Ec}	7.35 ± 0.13 ^{Fb}	6.20 ± 0.10 ^{Ea}
C+chitosan	9.29 ± 0.21 ^{E}	7.37 ± 0.10 ^{F}	6.82 ± 0.40 ^{G}
C- $NaHCO_3$	7.00 ± 0.04 ^{D}	5.67 ± 0.04 ^{B}	4.68 ± 0.34 ^{H}
C- NH_4HCO_3	9.12 ± 0.13 ^{E}	7.67 ± 0.15 ^{F}	6.89 ± 0.17 ^{G}
C- $NaHCO_3$- NH_4HCO_3	5.94 ± 0.08 ^{C}	5.85 ± 0.07 ^{B}	4.91 ± 0.17 ^{H}

Values are givens as mean ± standard deviation from triplicate determinations.
$^{A\text{-}H}$ Means in the same column with different superscript letters are significantly different ($p < 0.05$).
$^{a\text{-}c}$ Means in the same row with different superscript letters are significantly different ($p < 0.05$).
*a: Flour and water
a + sucrose: Flour, water and sucrose,
a + fructose: Flour, water and fructose,
a + glucose: Flour, water and glucose
a + NaCl: Flour, water and NaCl,
a + milk: Flour, water and nonfat dry milk
a + shortening: Flour, water and shortening
a + $NaHCO_3$: Flour, water and $NaHCO_3$
a + NH_4HCO_3: Flour, water and NH_4HCO_3
AACC (C): AACC formula
C + chitosan: AACC formula and chitosan
C - $NaHCO_3$: AACC formula without sodium bicarbonate addition
C - NH_4HCO_3: AACC formula without ammonium bicarbonate addition
C –$NaHCO_3$- NH_4HCO_3: AACC formula without leavening agent addition.

Table 3. Water activity of cookie and model cookies baked for 20 min

	Water activity		
	10 min	15 min	20 min
Flour	0.24±0.02 ^{Aa}	0.044±0.001 ^{Ab}	0.051±0.002 ^{ABb}
Flour+ water (a)	0.56±0.04 ^{BCDa}	0.039±0.004 ^{Ab}	0.042±0.001 ^{Ab}
a + sucrose	0.67±0.01 ^{Fa}	0.147±0.004 ^{Cb}	0.109±0.023 ^{Dc}
a + fructose	0.55±0.04 ^{BCDa}	0.296±0.005 ^{Fb}	0.304±0.016 ^{Gb}
a + glucose	0.67±0.09 ^{EFa}	0.207±0.024 ^{Eb}	0.178±0.032 ^{Eb}
a + NaCl	0.59±0.01 ^{CDEFa}	0.039±0.001 ^{Ac}	0.065±0.005 ^{ABb}
a + milk	0.58±0.07 ^{BCDEa}	0.052±0.006 ^{Ab}	0.079±0.005 ^{ABCDb}
a + shortening	0.64±0.06 ^{DEFa}	0.165±0.009 ^{Db}	0.105±0.007 ^{CDb}
a + $NaHCO_3$	0.49±0.03 ^{Ba}	0.048±0.008 ^{Ab}	0.084±0.030 ^{BCDb}
a + NH_4HCO_3	0.56±0.04 ^{BCDa}	0.128±0.004 ^{Bb}	0.070±0.030 ^{ABCb}
AACC(C)	0.51±0.05 ^{BCa}	0.174±0.007 ^{Db}	0.221±0.023 ^{Fb}
C+chitosan	0.54±0.01 ^{D}	0.192±0.009 ^{G}	0.197±0.048 ^{F}
C- $NaHCO_3$	0.52±0.01 ^{C}	0.236±0.012 ^{H}	0.202±0.032 ^{F}
C- NH_4HCO_3	0.48±0.01 ^{B}	0.235±0.006 ^{H}	0.195±0.011 ^{F}
C- $NaHCO_3$- NH_4HCO_3	0.54±0.02 ^{D}	0.238±0.015 ^{H}	0.199±0.015 ^{F}

Values are givens as mean ± standard deviation from triplicate determinations.
$^{A\text{-}H}$ Means in the same column with different superscript letters are significantly different ($p < 0.05$).
$^{a\text{-}c}$ Means in the same row with different superscript letters are significantly different ($p < 0.05$).
*a: Flour and water
a + sucrose: Flour, water and sucrose,
a + fructose: Flour, water and fructose,
a + glucose: Flour, water and glucose
a + NaCl: Flour, water and NaCl,
a + milk: Flour, water and nonfat dry milk
a + shortening: Flour, water and shortening
a + $NaHCO_3$: Flour, water and $NaHCO_3$
a + NH_4HCO_3: Flour, water and NH_4HCO_3
AACC (C): AACC formula
C + chitosan: AACC formula and chitosan
C - $NaHCO_3$: AACC formula without sodium bicarbonate addition
C - NH_4HCO_3: AACC formula without ammonium bicarbonate addition
C –$NaHCO_3$- NH_4HCO_3: AACC formula without leavening agent addition.

3.2. Acrylamide in Model Cookies and Cookies

The acrylamide concentration in cookies and model cookies (cake flour, water and fructose) for 10 min baking time was 178.2 ng/g and 150.6 ng/g, respectively (Table 3). For all the other model cookies, acrylamide concentration was not detected, or was less than 5.2 ng/g detected by the HPLC analytic method (Table 3). Cake flour and model cookies (cake flour with water) contained 647.6 ng/g and 790.1 ng/g acrylamide after 15 min baking time, which were higher levels than those of other model cookies (Table 3). However, they were still lower than the concentration (1129.0 ng/ng) of AACC cookies. Model cookies containing leavening agents, cake flour and water with sodium bicarbonate and ammonium bicarbonate, contained 458.6 ng/g and 163.4 ng/g acrylamide after 15 min baking time, indicating that ammonium bicarbonate would not induce the formation of acrylamide in model cookies (Table 3). The acrylamide content (73.0 ng/ng) in the model cookies (cake flour and water with shortening) had the lowest concentration compared to those of other model cookies after 15 min baking time (Table 3). Of the cookie ingredients used in this study, shortening mitigated the formation of acrylamide in model cookie baking; it might be due to the partial hydrogenation shortening preventing the reaction between amino acid and reducing sugar in the cake flour. Frideman and Mottram [31] propose that saturated fatty acid will generate less acrylamide compared to unsaturated fatty acid. Table 3 shows that the acrylamide concentrations of the model cookies with sucrose, fructose and glucose exhibited no significant difference after 20 min baking. It may indicate that the Maillard reaction was at the final stage, and the sucrose had already been converted to glucose and fructose. The results were different from that of Gokmen and Senyuva [32]; they reported that reducing sugar will increase the concentration of acrylamide.

The acrylamide concentration in the cookies without ammonium bicarbonate addition group was not detected by the HPLC analytic method after 10 min baking time (Table 3). The cookies without sodium bicarbonate, ammonium bicarbonate and both leavening agents contained 26.6%, 55.2% and 81.1% acrylamide compared to that of cookies, respectively, after 20 min baking time (Table 3). Cookies treated with ammonium bicarbonate contained more acrylamide, indicating that leavening agents induced acrylamide formation. However, the thickness of cookies and cookies without sodium bicarbonate, or ammonium bicarbonate, and cookies without both leavening agents decreased 17.7%, 0.1% and 18.7%, respectively, indicating that sodium bicarbonate was the main leavening effect for this cookie recipe. The acrylamide content in the model cookies (cake flour, water and ammonium bicarbonate) was significantly lower than that of AACC cookies, perhaps due to the AACC cookie recipe containing more sugar than that of the model cookie recipe. Among the leavening agents used in this study, ammonium carbonate was the most effective in terms of causing the formation of acrylamide. However, chitosan was only able to significantly mitigate the formation of acrylamide after 15 min baking time

(Table 3). Lindsay and Jang [11] explored the possibility of using ammoniated polymers such as chitosan for blocking the carbonyl groups of neutral reducing sugars on cut potato surfaces. They found that chitosan treatments used alone effected modest acrylamide reductions, and they proposed that chitosan was more effective when used in combination with other acrylamide reduction technologies [11].

3.3. Reducing Sugar in Model Cookies and Cookies

Adding sucrose, fructose and glucose into the model cookies showed a higher amount of reducing sugar in cookies and model cookies compared to other testing groups after 10 min baking time (Table 4). A trend of increase in reducing sugar was observed in cookies and model cookies from 10 min baking to 15 min baking time. Although the reducing sugar will react with amino acid during the Maillard reaction, some reducing sugar will be generated from the degradation of starch. Nevertheless, the amounts of reducing sugar in model cookies and cookies decreased after 20 min baking time, except for cake flour and water with shortening and sodium bicarbonate groups (Table 4); this may be due to the shortening slowing down the degradation of starch. Sucrose will be degraded into fructose and glucose, and starch will be degraded into maltose, glucose and dextrin during baking [29]. Leavening agents and chitosan did not significantly change the amount of reducing sugar in cookies during baking.

3.4. Correlation between the Content of Browning Index and Acrylamide

The browning indexes of baked model cookies were significantly lower than that in the cookies (Table 5). A higher browning index (35.03) was found for model cookies (cake flour and water with fructose) after 10 min baking as compared to the other model cookie groups (Table 5), which might contain higher amount of melanoidin, indicating that it was much easier for the fructose to generate the Maillard reaction compared to glucose and sucrose. However, the browning indexes of model cookie, cake flour and water, with the addition of fructose and shortening, were higher than those of the other model cookie groups after 10 min baking (Table 5). Therefore, higher browning index value was not only due to the Maillard reaction with the addition of keto and aldehyde groups in monosaccharides, but also shortening. Nevertheless, a higher browning index value of model cookies, cake flour and water, with the addition of shortening, did not show a high acrylamide concentration (Table 3). Table 5 shows that the browning index values increased with increasing baking time. Chitosan might have reacted with the reducing sugar and prevented the browning index value from increasing after 20 min baking time (Table 5).

Table 4. Acrylamide concentration of cookie and model cookies baked for 20 min

	Acrylamide (ng/g)		
	10 min	15 min	20 min
Flour	ND^{a}	647.62 ± 76.27^{Eb}	1410.87 ±28.72^{Fc}
Flour+ water(a)	ND^{a}	790.11 ± 93.05^{Fb}	953.42 ±26.77^{CDEc}
a + sucrose	ND^{a}	165.33 ± 37.27^{BCa}	964.21 ±215.33^{CEb}
a + fructose	150.64 ± 31.55^{Ba}	172.02 ± 40.60^{Ca}	907.83 ±108.49^{CDb}
a + glucose	ND^{a}	160.24 ± 1.24^{BCb}	1081.23 ±115.74^{DEc}
a + NaCl	ND^{a}	103.34 ± 4.03^{ABCa}	739.83 ±117.97^{BCb}
a + milk	ND^{a}	88.73 ± 14.87^{ABa}	618.50 ±136.58^{ABb}
a + shortening	ND^{a}	72.95 ± 7.79^{Ab}	408.63 ±41.28^{Ac}
a + $NaHCO_3$	ND^{a}	458.62 ± 7.95^{Db}	876.65 ±51.32^{CDc}
a + NH_4HCO_3	ND^{a}	163.36 ± 13.17^{BCb}	729.96 ±48.66^{BCc}
AACC(C)	178.19 ± 44.83^{aC}	1129.02 ± 37.78^{Gb}	1155.40 ±268.95^{Eb}
C+chitosan	149.71 ± 4.84^{B}	716.60 ± 30.76^{FE}	1075.05 ±287.59^{E}
C- $NaHCO_3$	108.75 ± 50.50^{A}	791.83 ± 114.71^{F}	848.71 ±253.43^{E}
C- NH_4HCO_3	ND	313.91 ± 23.16^{H}	517.62 ±30.09^{A}
C- $NaHCO_3$- NH_4HCO_3	ND	154.66 ± 14.98^{BC}	218.19 ±34.33^{G}

Values are givens as mean ± standard deviation from triplicate determinations.
$^{A-H}$ Means in the same column with different superscript letters are significantly different ($p < 0.05$).
$^{a-d}$ Means in the same row with different superscript letters are significantly different ($p < 0.05$).
*a: Flour and water
a + sucrose: Flour, water and sucrose,
a + fructose: Flour, water and fructose,
a + glucose: Flour, water and glucose
a + NaCl: Flour, water and NaCl,
a + milk: Flour, water and nonfat dry milk
a + shortening: Flour, water and shortening
a + $NaHCO_3$: Flour, water and $NaHCO_3$
a + NH_4HCO_3: Flour, water and NH_4HCO_3
AACC (C): AACC formula
C + chitosan: AACC formula and chitosan
C - $NaHCO_3$: AACC formula without sodium bicarbonate addition
C - NH_4HCO_3: AACC formula without ammonium bicarbonate addition
C –$NaHCO_3$- NH_4HCO_3: AACC formula without leavening agent addition.

Table 5. The reducing sugar of cookie and model cookies baked for 20 min

	Reducing sugar (%)		
	10 min	15 min	20 min
Flour	65.33 ± 4.50 [Aab]	73.10 ± 3.67 [Ab]	58.06 ± 7.63 [Aa]
Flour+ water(a)	76.05 ± 4.53 [Aa]	103.60 ± 2.71 [Cc]	90.58 ± 1.87 [BCb]
a + sucrose	116.16 ± 7.51 [Ba]	149.85 ± 1.75 [Ec]	136.30 ± 8.12 [Fb]
a + fructose	118.81 ± 15.62 [Ba]	133.18 ± 4.09 [Da]	126.68 ± 2.67 [EFa]
a + glucose	117.61 ± 13.44 [Ba]	150.59 ± 3.85 [Eb]	121.78 ± 4.67 [Ea]
a + NaCl	78.85 ± 14.26 [Aa]	106.99 ± 2.06 [Cb]	98.68 ± 9.58 [CDab]
a + milk	75.52 ± 9.03 [Aa]	103.03 ± 3.80 [Eb]	103.35 ± 6.57 [Db]
a + shortening	72.16 ± 6.65 [Aa]	80.92 ± 5.43 [Aa]	84.76 ± 8.84 [Ba]
a + $NaHCO_3$	78.93 ± 5.63 [Aa]	93.06 ± 9.84 [Bb]	93.28 ± 1.73 [BCDb]
a + NH_4HCO_3	75.53 ± 6.39[Aa]	102.30 ± 5.37 [BCb]	97.03 ± 2.86 [CDb]
AACC (C)	121.45 ± 14.24 [Ba]	142.34 ± 10.59 [DEa]	131.96 ± 3.48 [EFa]
C+chitosan	120.65 ± 6.25[B]	128.00 ± 3.44 [D]	131.17 ±3.24 [EF]
C- $NaHCO_3$	110.00 ± 8.00[B]	123.00 ± 0.02 [F]	132.87 ±11.39 [EF]
C- NH_4HCO_3	107.00 ± 7.00[B]	144.00 ± 0.10 [E]	122.52 ±3.15 [E]
C- $NaHCO_3$- NH_4HCO_3	115.00 ± 1.00[B]	129.00 ± 0.10 [D]	120.32 ±0.07 [E]

Values are givens as mean ±standard devieation from triplicate determinations.
[A-J] Means in the same column with different superscript letters are significantly different ($p < 0.05$).
[a-c] Means in the same row with different superscript letters are significantly different ($p < 0.05$).
*a: Flour and water
a + sucrose: Flour, water and sucrose,
a + fructose: Flour, water and fructose,
a + glucose: Flour, water and glucose
a + NaCl: Flour, water and NaCl,
a + milk: Flour, water and nonfat dry milk
a + shortening: Flour, water and shortening
a + $NaHCO_3$: Flour, water and $NaHCO_3$
a + NH_4HCO_3: Flour, water and NH_4HCO_3
AACC (C): AACC formula
C + chitosan: AACC formula and chitosan
C - $NaHCO_3$: AACC formula without sodium bicarbonate addition
C - NH_4HCO_3: AACC formula without ammonium bicarbonate addition
C –$NaHCO_3$- NH_4HCO_3: AACC formula without leavening agent addition.

The correlation between acrylamide concentration and the browning index value in model cookies and cookies was so low and it could not used to make predictions regarding the acrylamide level. Although the formation of acrylamide showed a positive relationship with the browning index (R^2=0.946) [1], it should be mentioned that the predicted relation between the formation of acrylamide level and browning index is based on various ingredients at different amounts in this study. Extremely higher browning index values of cookies were found in comparison to individual ingredients during baking (Table 5). Browning index values of model cookies with the addition of fructose and shortening demonstrated higher values compared to the other ingredients, but lower than the AACC cookie formulation after 10 min and 15 min baking (Table 5), possibly due to glucose, fructose and shortening enhancing the Maillard reaction during the first 15 min baking. However, the browning index value of the glucose group showed higher value, compared to the other ingredients, after 20 min baking, possibly due to the Maillard reaction in the fructose and shortening groups reaching the final stage after 15 min baking. Nevertheless, the Maillard reaction is still continuing in the model cookie group of flour, water and glucose formulation (Table 5) after 20 min baking. Overall, the browning index of cookie in cookie formulation was higher than that of the model cookie groups (Table 5). It showed that Maillard reaction and caramelization of the combination of ingredients would have a higher browning index value than that of model cookies, cake flour, water and the other ingredients.

Our results showed that the color of cookies and cookies without leavening agents became darker with increasing baking time. Table 5 shows that the browning index values of cookies without the addition of sodium bicarbonate groups were lower than those of cookies, cookies with chitosan, and cookies without the addition of ammonium bicarbonate after 10 min baking time. Cookies without the addition of leavening agents showed a lighter color and lower browning index values compared to the cookies after 15 min baking time (Table 5). The browning index value of cookies with the addition of chitosan was the lowest compared to the cookies and cookies without the addition of leavening agents after 15 min baking time (Table 5). However, there was no difference among the browning index values of all cookie groups after 20 min baking (Table 5). This may indicate that the Maillard reaction and caramelization are in the final stages after 20 min baking [33].

Concentrations of free amino acids produced by cookies and cookies without leavening agents for 15 min baking are shown in Table 6. Free asparagines in cookies and cookies without leaven agents for 15 min baking are 115.87 µM and 531.90 µM, respectively. Lower amounts of free asparagines in cookies may be due to their reacting with reducing sugar to form acrylamide. The acrylamide level of cookies without leavening agents (218.19 µM) is lower than that of cookies after 15 min baking time (1155.40µM) (Table 3). Furthermore, other free amino acids, including: alanine, arginine, glutamine, threonine and valine, can generate small amounts of acrylamide [8], in higher amounts in cookies without leavening agents (Table 6).

Table 6. The Browning index of cookie and model cookies baked for 20 min

	Browning index		
	10 min	15 min	20 min
Flour	9.42 ± 0.72 ^{Aa}	44.66 ± 0.59 ^{Db}	114.74 ± 2.05 ^{Dc}
Flour+ water(a)	14.70 ± 0.16 ^{Ba}	33.27 ± 0.53 ^{Cb}	109.34 ± 16.02 ^{CDc}
a + sucrose	14.71 ± 0.45 ^{Ba}	29.67 ± 1.42 ^{BCa}	90.347 ± 19.74 ^{BCb}
a + fructose	35.03 ± 4.20 ^{Da}	80.99 ± 5.28 ^{Fb}	70.50 ± 5.35 ^{Bc}
a + glucose	17.14 ± 2.24 ^{Ba}	82.59 ± 2.17 ^{Fb}	181.28 ± 17.89 ^{Ec}
a + NaCl	14.79 ± 2.07 ^{Ba}	26.69 ± 1.03 ^{Baa}	77.65 ± 18.69 ^{Bc}
a + milk	15.79 ± 2.23 ^{Ba}	21.69 ± 0.49 ^{Aa}	68.26 ± 10.49 ^{Bc}
a + shortening	44.60 ± 2.85 ^{Ea}	83.01 ± 3.60 ^{Fb}	101.33 ± 8.16 ^{CDc}
a + $NaHCO_3$	25.26 ± 3.02 ^{Ca}	58.39 ± 2.34 ^{Db}	73.94 ± 8.05 ^{Bc}
a + NH_4HCO_3	15.76 ± 1.71 ^{Ba}	20.62 ± 0.65 ^{Ab}	37.79 ± 3.06 ^{Ac}
AACC (C)	99.18 ± 5.88 ^{Fa}	218.19 ± 4.25 ^{Gb}	312.20 ± 13.24 ^{Fc}
C+chitosan	96.17 ± 5.56^{F}	81.59 ± 0.84 ^{F}	248.82 ±34.63 ^{G}
C- $NaHCO_3$	61.38 ± 1.85^{G}	192.60 ± 2.84 ^{H}	306.08 ±52.99 ^{GF}
C- NH_4HCO_3	103.89±9.90^{F}	185.22 ± 3.41 ^{I}	247.06 ±32.66 ^{G}
C- $NaHCO_3$- NH_4HCO_3	51.29±1.86 ^{H}	145.60 ± 2.58 ^{J}	262.94 ±52.24 ^{GF}

Values are givens as mean ± standard deviation from triplicate determinations.
$^{A-J}$ Means in the same column with different superscript letters are significantly different ($p < 0.05$).
$^{a-c}$ Means in the same row with different superscript letters are significantly different ($p < 0.05$).
*a: Flour and water
a + sucrose: Flour, water and sucrose,
a + fructose: Flour, water and fructose,
a + glucose: Flour, water and glucose
a + NaCl: Flour, water and NaCl,
a + milk: Flour, water and nonfat dry milk
a + shortening: Flour, water and shortening
a + $NaHCO_3$: Flour, water and $NaHCO_3$
a + NH_4HCO_3: Flour, water and NH_4HCO_3
AACC (C): AACC formula
C + chitosan: AACC formula and chitosan
C - $NaHCO_3$: AACC formula without sodium bicarbonate addition
C - NH_4HCO_3: AACC formula without ammonium bicarbonate addition
C –$NaHCO_3$- NH_4HCO_3: AACC formula without leavening agent addition

Table 7. Free amino acid concentration of cookies baked for 15 min

uM	AACC	AACC - $NaHCO_3$ - NH_4HCO_3
Asparagine	115.87	531.90
Aspartic acid	176.80	583.43
Glutamine	24.78	60.87
Alanine	125.46	285.02
Arginine	235.02	318.32
Cystine	1.59	5.53
Valine	19.82	138.44
Methionine	3.99	9.61
Threonine	50.14	163.28
γ-Aminobutyric acid	33.83	522.24
Hydroxylysine	22.93	19.42
1-Methylhistidine	7.40	4.153
Phosphoserine	4.18	3.35

Table 8. Thickness and expansion ratio of cookies baked for 20 min

	Thickness (cm)	Expansion ratio (%)
AACC	0.730 ± 0.010 ^{c}	100%
AACC + Chitosan	0.705 ± 0.017 ^{bc}	102.7%
AACC - $NaHCO_3$	0.629 ± 0.021 ^{a}	82.3%
AACC - NH_4HCO_3	0.664 ± 0.027 ^{ab}	99.9%
AACC - $NaHCO_3$- NH_4HCO_3	0.621 ± 0.051 ^{a}	81.3%

Mean ± standard deviation values with the different superscript letters in the same column are significantly different at $p < 0.05$. n=3
AACC: AACC formula
AACCC + chitosan: AACC formula and chitosan
AACC - $NaHCO_3$: AACC formula without sodium bicarbonate addition
AACC - NH_4HCO_3: AACC formula without ammonium bicarbonate addition
AACC –$NaHCO_3$- NH_4HCO_3: AACC formula without leavening agent addition.

3.5. Physicochemical Properties of Cookies, Model Cookies and Cookies without Leavening Agents

Model cookies of cake flour and water recipe with the addition of sodium bicarbonate, ammonium bicarbonate, milk powder or salt remained in powder form after 10 min baking. The model cookies of cake flour and water mixed with glucose or sucrose formed a lump shape and the dough was unable to be rolled into a sheet. Only cake flour and water with the addition of fructose and shortening were able to form a cookie shape after 10 min baking.

Cookies and model cookies with sodium bicarbonate had significant effects on cookie expansion (Table 7). Thickness of the model cookies was low due to the lack of leavening agents. In general, the diameter and thickness of cookies increased with adding leavening agents. As a result, the addition of sodium bicarbonate was most effective in increasing the expansion ratio of the cookies. The decrease in the expansion of cookies may be due to low pH causing low viscosity cookie dough to form, resulting in an enhanced cookie expansion ratio during the baking process.

4. Conclusion

The acrylamide content of model cookies, cake flour, water with sodium bicarbonate addition, was high compared to the other model cookies due to sodium bicarbonate inducing the Maillard reaction and generating more acrylamide in higher pH cookie dough. Although ammonium bicarbonate did not induce the formation of acrylamide in model cookies, it dramatically increased the acrylamide content in cookies due to its reaction with reducing sugar in the cookie formulation. Cookies without the addition of ammonium bicarbonate could mitigate the formation of acrylamide. Therefore, we suggest that only using sodium bicarbonate instead of sodium ammonium bicarbonate as the leavening agent in cookie formulation is a better strategy for baking cookies. Shortening may induce the formation of acrylamide in cookie formulation; however, it prevents the amino acid and reducing sugar from reacting in model cookies of flour and water in regard to the Maillard reaction during baking. Fructose would react with amino acid faster than sucrose and glucose to form acrylamide during the first 10 min baking time in model cookies; nevertheless, there would be no difference with 20 min baking compared to non-reducing sugar, sucrose. This indicates the final stage of the Maillard reaction after 20 min baking in the model cookies. Cookies without the addition of leavening agents would have a high concentration of free amino acid, especially for asparagine.

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