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ORIGINAL PAPER

Effect of Calcium on Acrylamide Level and Sensory Properties of Cookies

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Abstract Thermal process contaminants including acrylamide and hydroxymethylfurfural have been an intensive area of research in recent years. The main pathway of acrylamide formation is linked to the Maillard reaction. The first step is the formation of Schiff base between the carbonyl and α-amino group of asparagine. Presence of cations partially or completely eliminates the formation of Schiff base. This study aimed to investigate the effects of calcium chloride and calcium lactate on acrylamide and hydroxymethylfurfural levels in cookies. The effects of calcium derivatives on the sensory properties of cookies were also investigated. A direct relationship was determined between the amount of calcium in recipe and acrylamide formed in cookies. Addition of 1.0% of Puracal Act 100 decreased acrylamide concentration of cookies from 128±10 ng/g to 24±4 ng/g. In the same time, hydroxymethylfurfural concentration increased from 2.0± 0.19 mg/kg to 3.3±0.24 mg/kg by the addition of 1.0% of Puracal Act 100. The calcium derivatives had no effect on cookie diameter and thickness, but the surface colors were different. The use of calcium significantly increased the lightness (L*) parameter, but decreased the redness (a*; $p<0.05$). The sensory properties of cookies in terms of sweetness, saltiness and bitterness were not significantly affected by the addition of calcium derivatives at dosages up to 0.5% ($p>0.05$).

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Introduction

Baking is a complex process in which chemical and physical changes take place simultaneously. Dough pieces chiefly undergo changes in structure, taste, color, and size during baking. The Maillard reaction and caramelization are complex chemical reactions responsible for the formation of desirable taste and color of baked products (Gökmen et al. 2008).

Among many products formed, acrylamide and hydroxymethylfurfural, potentially harmful compounds seem to be particularly interesting because of their accumulation during the baking process. Hydroxymethylfurfural is formed as an intermediate product in the Maillard reaction, and is also formed from the caramelization of sugars at high temperatures (Kroh 1994). The main pathway of acrylamide formation in thermally processed foods is linked to the Maillard reaction, and in particular, to the presence of free asparagine (Mottram et al. 2002; Stadler et al. 2002). Processed cereals are among the foods in which acrylamide formation has been commonly encountered (EU JRC, 2006). These foods encompass a vast range of different products with many ingredients, processes, recipes, and scales of operation. Resulting acrylamide concentrations in these foods change with great deviations, not only for different types of bakery products, but also for a single type of product from different suppliers (EU JRC, 2006). Recent assessments by the Joint Expert Committee on Food Additives confirmed that a risk could not be excluded for dietary intake of acrylamide because it is classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC 1994).

Mitigation of acrylamide in thermally processed foods has been an intensive area of research shortly after the discovery of acrylamide in heated foods by Swedish researchers in April 2002 (Tareke et al. 2002). A number of possible mitigation strategies have been proposed for reducing acrylamide in bakery products. The most common strategies employed reduction or dilution of precursors (Sadd et al. 2008). The mitigation strategies of immediate practical application include reducing asparagine level by asparaginase (Ciesarova et al. 2006; Pedreschi et al. 2008), removing ammonium salts (Biedermann and Grob 2003; Amrein et al. 2006), replacing reducing sugars (Amrein et al. 2004; Gökmen et al. 2007a; b), adding glycine (Bråthen et al. 2005), and adding divalent cations (Lindsay and Jang 2005; Gökmen & Şenyuva 2007a). Decreasing thermal energy load by modifying the baking conditions has been shown to offer advantage for the mitigation of acrylamide in cookies (Gökmen et al. 2007a; b). These are by no means the complete or only mitigation strategies available to the baking industry.

The major concern of food producers is to reduce acrylamide content, but to keep quality parameters unaffected. Among the strategies proposed so far, use of divalent cations like calcium brings not only the mitigation of acrylamide, but also nutritionally supplement bakery products. However, utilization of calcium derivatives in different products needs to be investigated in details whether they adversely influence the sensory properties or cause the formation of harmful compounds like hydroxymethylfurfural in bakery products. Because model study previously revealed that cations may increase furfurals while decreasing acrylamide (Gökmen & Şenyuva, 2007b).

This study aimed to investigate the effects of calcium chloride and calcium lactate on the formation of acrylamide and hydroxymethylfurfural in cookies during baking. A cookie model was selected in the basis that cookie resembling bakery foods are widely consumed and are among the foods in which acrylamide formation has been widely encountered. The effects of calcium derivatives on the sensory properties of cookies were also investigated in this study.

Materials and Methods

Chemicals and Consumables

Acrylamide (99+%) and hydroxymethylfurfural were purchased from Sigma (Deisenhofen, Germany). Formic acid, acetic acid, calcium chloride, 9-fluorenylmethylchloroformate (FMOC), potassium hexacyanoferrate, and zinc sulfate (all AnalaR grade) were purchased from Merck (Darmstadt, Germany). Gradient grade acetonitrile was purchased from J.T. Baker (Deventer, Holland). Ultra pure water was used throughout the experiments (MilliQ system, Millipore, Bedford, MA, USA). Oasis MCX (1 ml, 30 mg) solid phase extraction (SPE) cartridges, Atlantis dC18 column (4.6× 300 mm, 5 μm) and Atlantis T_3 analytical column (4.6× 150 mm, 3 μm) were supplied by Waters (Milford, MA, USA). Zorbax C8 (150×4.6 mm) column was purchased from Agilent Technologies (Palo Alto, CA, USA).

The calcium salts of lactic acid (Puracal Act 100 and Puracal Act 200) were obtained from Purac (Netherlands). The ratio of calcium and lactate were 23% and 35% by weight for Puracal Act 100 and 20% and 44% by weight for Puracal Act 200, respectively. These salts are naturally produced by fermentation of sugar and calcium chloride. Wheat flour and food grade baking ingredients (sodium bicarbonate, salt, shortening, non-fat dry milk, sucrose, brown sugar, high fructose corn syrup) were kindly supplied by a baking company Eti (Eskişehir, Turkey).

Preparation of Cookie Samples

Cookies were prepared as described in American Association of Cereal Chemists (AACC) method 10-54 (AACC 2000). The base recipe was formulated with 80 g of wheat flour, 32 g of shortening, 1 g of salt, 17.6 g of deionized water, 0.4 g of sodium bicarbonate, 0.8 g of ammonium bicarbonate, 25.6 g of non-fat dry milk, 25.6 g of sucrose, 8.0 g of brown sugar, and 1.2 g of high fructose corn syrup. The base recipe was modified by the addition of Puracal Act 100, Puracal Act 200, and $CaCl_2$ to determine the effects of calcium derivatives on acrylamide formation. Preliminary experiments showed that amount of calcium derivative exceeding 1.0% adversely affected visual quality of cookies. Therefore, three levels of calcium derivatives (0.1%, 0.5%, and 1.0% in flour weight basis) corresponding to the amounts of 0.08, 0.4, and 0.8 g were added to dough. Calcium derivatives were first dissolved in water, and added to dough. The dough was prepared by mixing the ingredients. It was rolled out to obtain the disks having a diameter of 6 cm with a thickness of 1 cm. The disks were baked in an oven (Memmert UNE 400, Germany) at 205 °C for 11 min as recommended in AACC method 10-54 (AACC 2000).

The effect of baking temperature (150, 200, and 250 °C) and time (3-60 min) were also investigated. In order to see the effect of calcium on the kinetics of acrylamide formation, cookie dough formulated with 1.0% calcium chloride was used. The disks were baked for 10, 30, and 60 min at 150 °C, for 5, 10, and 15 min at 200 °C, and 3, 5, and 8 min at 250 °C. Two disks were baked in parallel for each baking conditions (n=4).

Acrylamide Analysis

Acrylamide was analyzed by liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) using the method described elsewhere

(Gökmen et al. 2009). A finely ground cookie sample (1 g) was extracted with 2×10 ml of 10 mM formic acid by mixing in a vortex mixer for 2 min. The co-extracted colloids were precipitated by means of Carrez I and Carrez II reagents. The fat was separated by means of cold centrifugation at 10,000×*g* for 10 min (0 °C). A cation exchanger-based SPE was used to clean up the extract. The supernatant was eluted through a preconditioned Oasis MCX cartridge at a rate of one drop per second. First seven to eight drops of the eluate were discarded, while the remaining drops were collected and filtered through 0.45 μm nylon filter prior to analysis.

LC-APCI-MS analyses were performed by an Agilent 1200 HPLC system (Waldbronn, Germany) consisting of a binary pump, an autosampler, and a temperature-controlled column oven, coupled to an Agilent 6130 MS detector equipped with multimode interface using the following interface parameters: drying gas (N_2, 20 psig) flow of 5 L/min, nebulizer pressure of 20 psig, drying gas temperature of 350 °C, capillary voltage of 2,000 V, and corona current of 5 μA. The analytical separation was performed on a Atlantis T_3 column (150×4.6 mm, 3 μm) using the isocratic mixture of 10 mM formic acid at a flow rate of 0.3 ml/min at 25 °C. The LC eluent was directed to the MS system from 10 min to 16 min using Agilent Chemstation software. Ions monitored were *m/z* 72 and 55 for the quantification of acrylamide in the samples. Acrylamide concentrations were calculated from the calibration curve that built with standard solutions of acrylamide dissolved in Milli-Q water (5, 10, 20, 50, and 100 μg/l). All analyses were performed in triplicate and the results expressed as ng/g sample.

Hydroxymethylfurfural Analysis

Hydroxymethylfurfural (HMF) was analyzed by high-performance liquid chromatography (HPLC) using the method of Gökmen and Şenyuva (2006). An Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a quaternary pump, an autosampler, a diode array detector, and a temperature-controlled column oven was used. The chromatographic separations were performed on an Atlantis dC18 column, using the isocratic mixture of 0.1% aqueous acetic acid solution and acetonitrile (90:10, *v/v*) at a flow rate of 1.0 ml/min at 40 °C. Data acquisition was performed, acquiring chromatograms at the detection wavelength of 285 nm. HMF concentrations were calculated from the calibration curve that is built with standard solutions of HMF dissolved in Milli-Q water (0.05, 0.1, 0.2, 0.5, and 1.0 μg/ml). All analyses were performed in triplicate and the results expressed as mg/kg sample.

Asparagine Analysis

The initial free asparagine level of wheat flour was determined to be 260±21 mg/kg (n=2) using HPLC method (Gökmen et al. 2007a; b). Asparagine was converted to its FMOC derivative prior to HPLC analysis. An Agilent 1100 system consisting of a quaternary pump, a Rheodyne 7125 injector, and a fluorescence detector set at 265 nm/340 nm was used. Chromatographic separation was performed on a Zorbax C8 column using a gradient mixture of acetonitrile (A) and 50 mM, pH 4.2, aqueous acetate buffer (B) at a flow rate of 1.0 mL/min at 25 °C. Acetonitrile ratio was increased from 0% to 25% within 10 min and to 100% within 5 min.

Sensory Analysis

In order to measure the perceived gustative differences between the nine samples prepared with different amounts (0.1%, 0.5%, and 1.0%) of calcium chloride and calcium lactate (Puracal 100 and Act 200) and the control sample, the difference from control test was performed (Meilgaard et al. 1999) with 15 untrained subjects as to reflect consumers' perception. In order to avoid that subjects evaluated the differences between the samples and the control on the base of visual attributes, cookies were reduced to crumbs before the tasting and the test was performed under red light to score sweetness, saltiness, and bitterness attributes. The surface colors were separately analyzed by digital image analysis. The samples consisting of half parts of cookies were served in plastic cups identified by three-digit random codes. They were presented simultaneously to the assessors together with the control sample marked with R. Among the samples, a blind control sample was also presented and it was indicated to the assessors that some of the samples might be the same as the control. The assessors were provided with a spoon to taste the samples. They were asked to rate the size of the difference between each sample and the control on a line scale ranging from 0 (no difference) to 10 (extremely different). Sensory data were collected over four sessions (n=18): the samples were split into two blocks and each sample was tested following a randomized design with two replications. The data were collected by means of "FIZZ Acquisition" software (Biosystèmes, Couternon, France).

Color Analysis

Color measurements (CIE $L^*a^*b^*$ parameters) were performed using a procedure described elsewhere (Gökmen & Süğüt, 2007). The total color change (ΔE) was calculated by the following formula;

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2}$$

where L_0^*, a_0^*, and b_0^* correspond to the CIE color parameters of cookie dough, whereas L^*, a^*, and, b^*

correspond to the CIE color parameters of cookies baked at different conditions.

Digital images were taken from an image acquisition system consisting of a digital camera placed vertically at a distance of 25 cm from the sample. The angle between the axes of the lens and the sources of illumination is approximately 45°. Illumination was achieved with two Philips Natural Daylight 18 W fluorescent lamps with color temperature of 6,500 K. Images were captured at a resolution of 5.0 megapixel and stored in a personal computer in jpeg format without compression. Two cookie disks were analyzed for each sample. Mean values and standard deviations were reported.

Statistical Analysis

The sensory test results were analyzed by means of analysis of variance (ANOVA) and Dunnett's test for multiple comparisons with a blind control was applied to the sample means (SPSS v. 11.5, SPSS Inc., Chicago, IL, USA). The samples resulting significantly different ($p \leq 0.05$) from the blind control were submitted to paired comparison tests in order to compare them with the control in terms of sweetness, saltiness and bitterness.

Acrylamide and HMF data were also subjected to ANOVA test for the evaluation of statistical significance of the differences between mean values by Tukey's test and for the calculation of Pearson correlation coefficients between different variables.

Results and Discussion

Acrylamide concentration of control cookie baked at 205 °C for 11 min according to the recommendation of AACC was determined to be 128±10 ng/g. Figure 1 shows the effects of different amounts of various calcium derivatives on the resulting acrylamide concentrations of cookies. The use of calcium derivatives decreased acrylamide formation in cookies. A direct relationship was determined between the amount of calcium in recipe and acrylamide formed in cookies during baking at 205 °C for 11 min. The amount of calcium derivative significantly influenced acrylamide formation in cookies ($p<0.05$). There was a sharp increase in the rate of acrylamide reduction when the amount of ingredient was increased from 0.1% to 0.5% in the recipe. For the amounts of 0.5% and 1.0% in recipe, Puracal Act 100 and $CaCl_2$ were found more effective ingredients to mitigate acrylamide formation in cookies than Puracal Act 200 ($p<0.05$). Acrylamide concentration of cookies formulated with 0.5% of Puracal Act 100 and $CaCl_2$ were determined to be 35±8 ng/g and 43±6 ng/g, respectively. In comparison to control, these acrylamide levels indicated

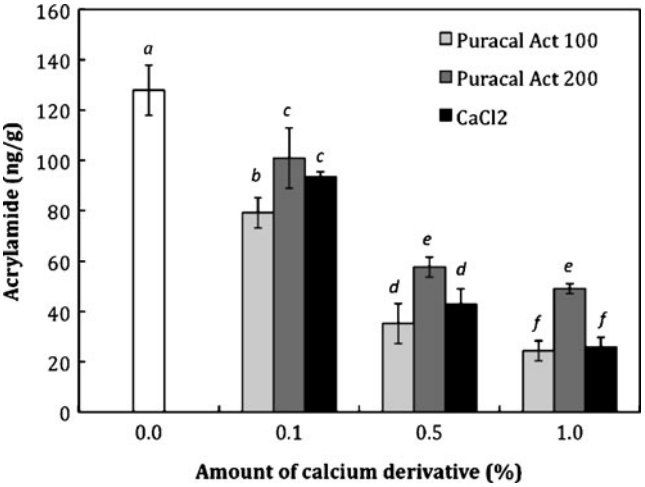

Fig. 1 Effect of different amounts of calcium derivatives on acrylamide formation in cookies baked at 205 °C for 11 min. *Different letters* indicate significant differences in acrylamide concentrations ($p<0.05$)

72.4% and 66.3% decrease for Puracal Act 100 and $CaCl_2$, respectively. When the amount of Puracal Act 100 was increased to 1.0%, the percentage reduction of acrylamide increased to 81.25% in cookies. Among the calcium derivatives tested in this study, Puracal Act 200 was found as the least effective ingredient in terms of the reduction of acrylamide formation. However, it significantly decreased acrylamide formation in cookies comparing to control when added to formulation at amounts ranging between 0.1% and 1.0% ($p<0.05$). Comparing to Puracal Act 100, Puracal Act 200 was composed of lower amount of calcium and higher amount of lactate. It has been reported previously that addition of organic acid into cookie formulation could slightly increase acrylamide formation due to hydrolysis of sucrose (Gökmen et al., 2007a; b).

It has been recently suggested that adding divalent metal ions could give high-temperature stability to asparagine/matrix interactions, thereby rendering the latter species unavailable for reaction with carbonyl precursors to produce acrylamide (Gökmen & Şenyuva, 2007a). Sadd et al. (2008) have reported that calcium fortification of dough in different forms is capable of reducing acrylamide level in bread. The standard calcium fortification of 0.3% required by UK law for nutritional reasons was found useful giving a significant reduction in acrylamide level in breads (Sadd et al. 2008).

In this study, experimental cookies with and without calcium fortification were prepared by baking at 150 °C (10, 30, and 60 min), 200 °C (5, 10, and 15 min) and 250 °C (3, 5, and 8 min) to determine the effect of calcium on the rate of acrylamde formation. These baking conditions allowed to obtain cookies with acceptable surface browning and interior. In general, increasing baking temperature and time also

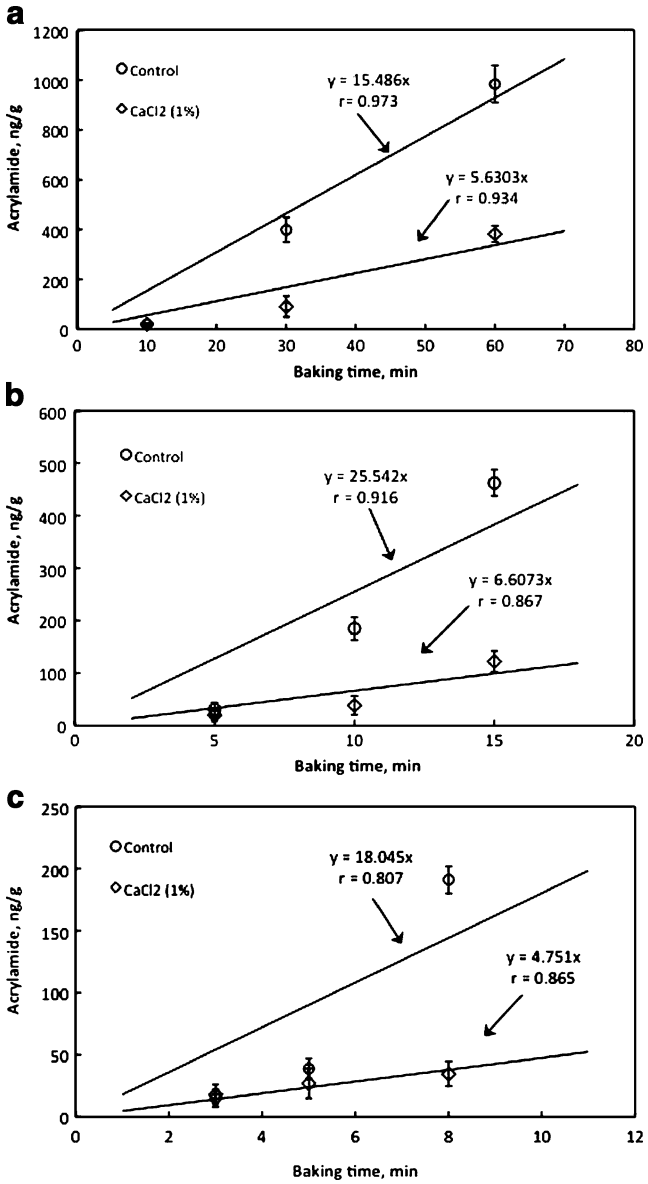

Fig. 2 Effect of calcium chloride on the kinetics of acrylamide formation in cookies during baking at **a** 150 °C, **b** 200 °C, and **c** 250 °C

increased browning. Figure 2 shows the change of acrylamide concentrations with time in cookies at different temperatures. Increasing baking temperature also increased the amount of acrylamide formed in cookies. Baking at 150 °C for 10 min formed 20±4 ng/g acrylamide in cookies. It increased to 185±22 ng/g in cookies baked at 200 °C for 10 min and to 191±11 ng/g in cookies baked at 250 °C for 8 min. Temperature and time are considered two of significant co-variants on acrylamide formation. Under the baking conditions investigated in this study, acrylamide formation in cookies followed a zeroth order kinetic pattern. Reaction rates (slopes shown in Fig. 2) were used to compare the effect of calcium chloride (1.0%) on acrylamide formation in cookies. The prevention of acrylamide formation in the presence of 1.0% $CaCl_2$ were calculated as follows:

$$\text{Prevention } (\%) = \left(1 - \frac{\text{rate } (CaCl_2)}{\text{rate } (\text{control})}\right) \times 100$$

Comparing to control, the results indicated that the presence of $CaCl_2$ significantly prevented acrylamide formation in cookies during baking at all temperatures ($p<0.05$). The inhibition ratios were found to be 63.65%, 74.13%, and 73.67% at 150, 200, and 250 °C, respectively.

Acrylamide formation in foods obeys a typical kinetic pattern during heating (Gökmen and Palazoğlu 2008). The initial period of baking is characterized by rapid loss of moisture, but the water activity is usually higher than 0.4 in this period (Gökmen et al. 2007a; b). Acrylamide concentration in foods tends to increase to an apparent maximum and then decreases exponentially. Since baking temperature may significantly differ for various bakery products, it is essential to see the effect of calcium fortification on acrylamide formation over a range of temperature and time.

The effect of calcium fortification on the formation of HMF was also determined in this study. Figure 3 shows the effects of different amounts of various calcium derivatives on the resulting HMF concentrations of cookies baked at 205 °C for 11 min. The HMF concentration of control cookies was found to be 1.97±0.2 mg/kg. The maxium HMF concentration was determined to be 4.2±0.2 for cookie fomulated with 0.5% of $CaCl_2$. This level of HMF in cookies is still too low from a food safety point of view. The Codex Alimentarius Commission has established that HMF concentration should be less than 40 mg/kg in honey (CODEX 2001).

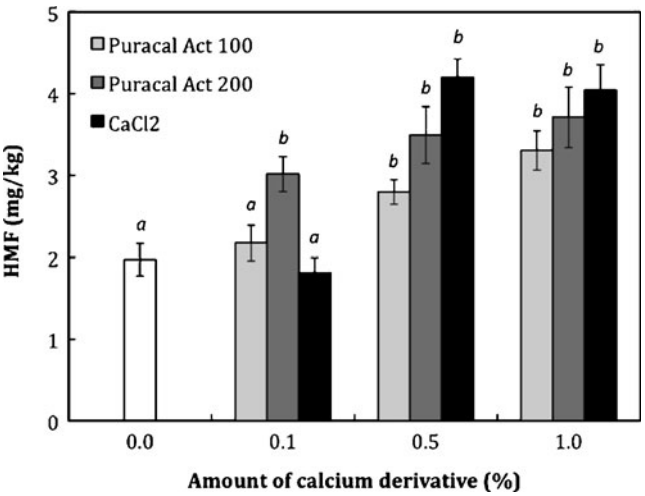

Fig. 3 Effect of different amounts of calcium derivatives on HMF formation in cookies baked at 205 °C for 11 min. Different letters indicate significant differences in acrylamide concentrations ($p<0.05$).

Table 1 CIE $L^*a^*b^*$ values of cookies formulated with different amounts of various calcium derivatives as the mitigation agent

	L^*	a^*	b^*	ΔE
Control	28.8±0.4[a]	19.0±0.0[a]	33.2±0.4[a]	0
Puracal Act 100				
0.1%	39.7±0.1[b]	12.4±0.1[b]	36.3±0.2[b]	13.1
0.5%	39.0±1.1[b]	13.1±0.2[b]	36.5±0.5[b]	12.3
1.0%	34.4±2.4[b]	15.8±1.3[b]	35.4±1.4[b]	6.8
Puracal Act 200				
0.1%	35.4±0.4[b]	17.5±0.4[a]	37.1±0.0[b]	7.8
0.5%	38.4±1.0[b]	14.5±0.3[b]	37.3±0.8[b]	11.4
1.0%	38.8±0.3[b]	13.6±0.6[b]	37.1±0.6[b]	12.1
$CaCl_2$				
0.1%	37.8±0.6[b]	15.6±0.6[b]	37.5±0.9[b]	10.6
0.5%	46.6±1.4[c]	10.5±0.5[b]	37.0±0.7[b]	20.1
1.0%	49.1±0.6[c]	7.5±0.1[c]	34.4±0.2[a]	23.4

ΔE total color difference

Values followed by a different superscript letters are significantly different ($p<0.05$) among each parameter

Comparing to control, use of calcium derivatives at amounts higher than 0.1% significantly increased HMF concentration in cookies ($p<0.05$). For the amount of 0.1%, Puracal Act 100 and $CaCl_2$ were found similar to control, but Puracal Act 200 differed significantly. HMF concentrations of cookies formulated with 0.5% and 1.0% of Puracal Act 100 were determined to be 2.8±0.15 mg/kg and 3.3±0.24 mg/kg, respectively, which were lower than those of cookies formulated with Puracal Act 200 and $CaCl_2$. However, the type of calcium derivative had no significant effect ($p>0.05$) on the resulting HMF concentration of cookies when they were added at amounts of 0.5 and 1.0% (Fig. 3).

Mitigation of acrylamide in foods by means of calcium derivatives requires a careful consideration because any change in the formulation may have adverse adverse effects in terms of the formation of other process contaminants such as HMF. It has been recently reported that the presence of cations reduces acrylamide formation, but increases HMF and furfural formation during heating. There is evidence that the cations effectively prevent the formation of Schiff base, which is the key intermediate leading to acrylamide, and mainly change the reaction path toward the dehydration of glucose leading to HMF and furfural (Gökmen and Senyuva 2007b).

Fortification of cookies with different calcium derivatives had no significant effect on cookie diameter and thickness. However, there were differences in the surface color values of cookies as determined in CIE $L^*a^*b^*$ units. Table 1 gives the $L^*a^*b^*$ values of cookies formulated with different calcium derivatives. In general, addition of calcium derivatives into cookie formulation increased surface lightness (L*), while surface yellowness only slightly increased (b*) in some samples. Meanwhile, redness (a*) parameter decreased by means of the addition calcium derivatives. In comparison to control, CIE $L^*a^*b^*$ values of cookies formulated with Puracal Act 100, Puracal Act 200 and $CaCl_2$ were significantly different ($p<0.05$) as given in Table 1. Comparing calcium derivatives for their influence on total color difference of cookies, calcium chloride was the most effective ingredient. In general, cookies formulated with different amounts of Puracal Act 200 had surface color values comparable to those of control. However, in most of the samples the differences were significant.

Effects of Calcium Derivatives on the Sensory Properties

Figure 4 shows the effects of different concentrations of various calcium derivatives on the sensory differences between the samples and the control. From a sensory point of view, the addition of 0.1% and 0.5% of Puracal Act 100, Puracal Act 200 and $CaCl_2$ in the recipe resulted in cookies not significantly different from the control sample in terms of sweetness, saltiness, and bitterness. Instead, at dosage of 1.0%, all the calcium derivatives in formulation were sensory perceived, in fact cookies prepared with Puracal Act 200 were significantly different ($p<0.01$) from the control sample while cookies prepared with Puracal Act 100 and calcium chloride were extremely different ($p<0.001$) from control sample. The fact that cookies prepared with Puracal Act 200 was less different than those prepared with Puracal Act 100 from the control sample, could be due to the different calcium content (20% and 23%, respectively).

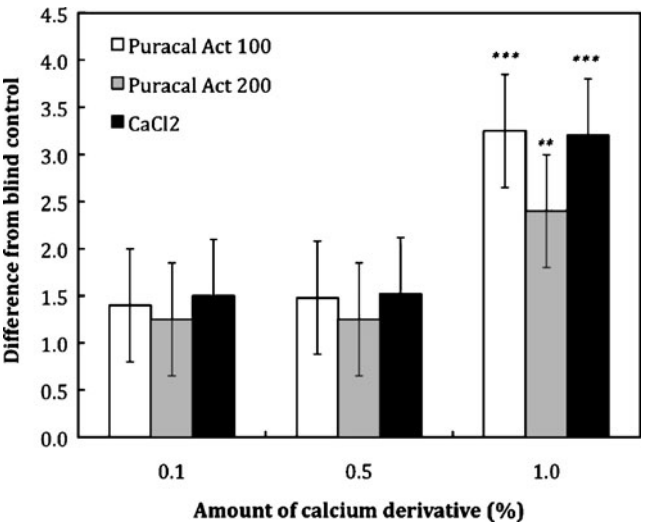

Fig. 4 Differences of mean ratings (± standard error) of each sample from blind control. Zero level = no difference between a rating of a sample vs. blind control; $^{**}p<0.01$, $^{***}p<0.001$

This result agreed with the results reported by Mestdagh et al. (2008). They studied the effect of several components on sensory quality of potato crisps when added to the blanching water. They found that at 0.1 M a bitter aftertaste was perceived in the calcium chloride treated products, which was not detected by the panelists at the 0.05 M. Similarly, Puracal Act 100 and 200 were accepted at concentration level of 0.04 and 0.03 M calcium, respectively.

In general, they found that calcium addition provoked a more crispy texture, compared to the control sample. In this study, texture evaluation of the samples was not considered, thus further study is necessary to understand as calcium derivatives addition affects cookies texture profile.

The cookies prepared with all the calcium derivatives at dosage of 1.0% were submitted to three paired comparison tests in order to evaluate if they were perceived as different from the control sample in terms of sweetness, saltiness, and bitterness. Table 2 gives paired comparison tests results. In particular, cookies prepared with Puracal Act 100 and with calcium chloride were more salt and less sweet than control sample, respectively. No difference was found between cookies prepared with Puracal Act 200 and control sample in terms of sweetness, saltiness, and bitterness. Probably, the dissimilarity detected by means of the difference from control test were due to other sensory attributes not investigated in this study.

The addition of calcium derivatives in cookies recipe did not cause a bitterness increase. This result disagreed with other studies findings (Mestdagh et al. 2008). Lawless et al. (2003) examined whether mixtures with other simple tastants would modify the tastes of calcium chloride. They found that alone calcium chloride was bitter, whereas mixtures with sucrose, citric acid, or sodium chloride suppressed bitterness of calcium chloride. Suutarinen et al. (2002) found bitter off-flavor in strawberry jams treated with calcium chloride.

Conclusion

In conclusion, utilization of calcium derivatives was found as a viable approach for the mitigation of acrylamide in cookie resembling bakery products. Both the type and amount of calcium derivative appeared as effective parameters from the viewpoint of acrylamide formation. These parameters were also found effective on HMF formation, surface color, and sensory properties under certain conditions. The adverse effects of mitigation strategies on sensory properties are considered as a significant limitation for their applicability in the food industry.

Puracal Act 100 and $CaCl_2$ are two of commercially available calcium derivatives, which seem to reduce acrylamide formation in cookies significantly. When these compounds are added to dough at amount of 0.5%, it is possible to reduce acrylamide formation up to 70% without any significant change in sensory properties of cookies. One of points to be considered during the modification of cookie recipe is the increased formation of HMF when calcium derivatives are added. The results of this study indicated that HMF levels of cookies were still too low (<5.0 mg/kg) when cookies were formulated with calcium derivatives at amounts ranging between 0.1% and 1.0%.

Baking conditions significantly differs for different kinds of bakery products. Therefore, it is important to see the effect of calcium in a range of baking conditions. It was clear from the results of this study that increasing baking

Table 2 Paired comparison test results

	Sample	Answers (correct verdicts)	Significance
Sweetness	Control Puracal Act 100 1%	27 21	0.471
Saltiness	Control Puracal Act 100 1%	16 32	**0.029**[a]
Bitterness	Control Puracal Act 100 1%	23 25	0.885
Sweetness	Control Puracal Act 200 1%	26 27	> 0.999
Saltiness	Control Puracal Act 200 1%	24 29	0.583
Bitterness	Control Puracal Act 200 1%	29 24	0.583
Sweetness	Control $CaCl_2$ 1%	32 16	0.029[a]
Saltiness	Control $CaCl_2$ 1%	20 28	0.312
Bitterrness	Control $CaCl_2$ 1%	19 29	0.193

[a] The difference was significant

temperature also increased the amount of acrylamide formed in cookies during baking. However, the rate of acrylamide formation was significantly decreased in the presence of calcium at all baking temperatures ranging between 150 and 250 °C.

Finally, it is considered that bakery foods can be composed of micronutrients like calcium and zinc not only for a fortification purpose, but also to limit the formation of acrylamide.

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