The effect of salts on acrylamide and 5-hydroxymethylfurfural formation in glucose-asparagine model solutions and biscuits

BARBARA QUARTA – MONICA ANESE

Summary
Increasing amounts of CH_3COOK, $CaCl_2$, $MgCl_2$ or of a combination of $CaCl_2$ and $MgCl_2$ were added to a glucose-asparagine model solution or to a biscuit formulation, and their effects on acrylamide and 5-hydroxymethylfurfural (HMF) formation were studied. The results showed that in both considered systems, CH_3COOK was responsible for a great increase in acrylamide (116% in the biscuits), while it inhibited HMF formation in both. On the contrary, $CaCl_2$ and $MgCl_2$ were effective in reducing acrylamide formation in the model solutions and, only if used in combination, they caused a 60% acrylamide reduction in the biscuits. However, these salts resulted in favouring the development of high levels of HMF. It was concluded that although the addition of some salts to foods has been suggested as a tool for reducing acrylamide formation, alternative interventions should be considered.

Keywords
acrylamide; 5-hydroxymethylfurfural; cations; mitigation; formation; biscuits; model solutions

One of the most important objectives of the food industry is to develop safe and healthy products for the customers. Nevertheless, in the last years, the attainment of this goal was made difficult by the discovery of toxic molecules in several heat-processed foods, such as acrylamide and furans, namely 5-hydroxymethylfurfural (HMF) [1–7].

Acrylamide has been recognized as potentially carcinogenic for humans by the International Agency for Research on Cancer [8]. Acrylamide in foods largely results from the Maillard reaction between the amino acid asparagine and a reactive carbonyl group, proceeding through intermediates that include a Schiff base [9]. The highest levels of acrylamide have been found in French fries, potato chips, and other fried, deep-fat fried or oven-cooked potato products, together with some crisp bread, breakfast cereals, biscuits and crackers [4, 5].

HMF is suspected to have genotoxic and mutagenic effects [10]. In vitro it can be bioactivated and transformed to the mutagenic 5-sulphoxymethylfurfural [11]. However, at present, neither chronic carcinogenic studies nor epidemiological data on potential association of HMF with cancer risk in humans are available [12, 13]. HMF is generated under heating by Maillard reaction or reducing sugar caramelization, which undergo 1-2 enolization, dehydration and cyclization reactions [14, 15]. HMF occurs in the volatiles of all heated foods and is considered an indicator of excessive thermal treatment.

Due to the toxicity and potential carcinogenity of these heat-induced molecules, efforts have been carried out to find technological strategies able to minimize their formation in foods. These include pre-treatments, modification of the process conditions and/or formulation changes. Among the latter, the potential of cations in reducing acrylamide levels has been studied. KOLEK et al. [16–18] observed that the addition of NaCl to glucose-asparagine model solutions had an inhibiting effect on acrylamide formation, and suggested that the salt could accelerate acrylamide elimination by decreasing the starting temperature of acrylamide polymerization. An inhibiting effect of monovalent as well as divalent and trivalent cations on acrylamide formation was also observed in glucose- or fructose-asparagine model solutions, potato model systems, fried potatoes and potato strips [19–23]. In all cases, the monovalent cations (Na^+, K^+) were less effective in reducing acrylamide formation than the divalent or trivalent ones (Ca^{2+},

Barbara Quarta, Monica Anese, Dipartimento di Scienze degli Alimenti, University of Udine, Via Sondrio 2/A, 33100 Udine, Italy.

Correspondence author:
Barbara Quarta, e-mail: barbara.quarta@uniud.it

Mg^{2+}, Zn^{2+}, Fe^{3+}). The supposed mechanism was that cations may increase the rate of glucose decomposition, while most asparagine remains unreacted, thus preventing the formation of the Schiff base and consequently of acrylamide [19]. Alternatively, according to reports by O'Brien and Morrissey [24] and Delgado-Andrade et al. [25], divalent and trivalent cations might form complexes with amines and some intermediates of the Maillard reaction, such as acrylic acid, the latter being a recognized precursor for acrylamide formation [26, 27]. The effect of cations on acrylamide formation was investigated also in cereal model systems [28, 29]. These authors reported an inhibiting effect of monovalent and divalent cations on acrylamide formation, $CaCl_2$ being the most effective salt.

In light of these results, the addition of Ca^{2+} or Mg^{2+} has been recently suggested as a possible intervention to minimize acrylamide formation in certain bakery products and potato derivatives [30]. However, it must be pointed out that the use of some salts may be responsible for undesired effects, such as failure in the development of desired sensory properties as well as possible opposite effects on acrylamide and HMF formation. For instance, studies on the effect of NaCl in both model systems and foods (crackers, wheat bread) have shown that relatively low Na^{+} concentrations (1–2% w/w) decreased acrylamide formation, whereas at higher levels of salt, the toxic molecules were formed [16, 31, 32]. When added in the amounts of 1–2% w/w to cracker and sweet doughs, calcium chloride caused an approximately 60% reduction in acrylamide contents, but it was also responsible for the failure of the dough to rise and for consequent unacceptability of the product [33]. By contrast, calcium propionate promoted acrylamide formation in the range of 0.35–0.75% (w/w). Gökmen and Senyuva [20] found that against a great reduction in acrylamide formation, the addition of Ca^{2+}, Mg^{2+} and Fe^{3+} to a glucose-asparagine model solution was responsible for the development of HMF and furfural, due to the dehydrating effect of salts towards glucose.

Therefore, the aim of this work was to investigate the effect of monovalent and divalent cations on acrylamide and HMF development in glucose-asparagine model solutions and short-dough biscuits, also in relation to possible water activity (a_w) and pH changes due to the salt addition. As is well known, water activity and pH may influence the reactivity of chemical constituents of a food matrix and could play an important role in the mechanisms of acrylamide and HMF formation. Although only few papers deal with the influence of a_w on acrylamide formation, different and sometimes contradictory results have been obtained [34–37]. On the contrary, it is well known that acrylamide formation is promoted by high pH values, due to the increased reactivity of both the carbonyl group of the reducing sugar and the asparagine amino group [38, 39]. With regard to HMF, it has been demonstrated that its formation is favoured by intermediate-high a_w [40] and low pH values [41, 42].

Short-dough biscuits were chosen for this study because they represent a worldwide consumed food. Besides that, studies on the effect of salts on the simultaneous development of acrylamide and HMF in this food matrix are not available. CH_3COOK, $CaCl_2$, $MgCl_2$ and a mixture of $CaCl_2$ and $MgCl_2$ were used in the study. These salts are frequently used in bakery product manufacturing. In particular, CH_3COOK (E 261) may be added to prevent the dough collapse, while $CaCl_2$ and $MgCl_2$ (E509 and E511, respectively) may be used in baked goods for product preservation and calcium fortification.

MATERIALS AND METHODS

Preparation of the model solutions

Increasing amounts of CH_3COOK, $CaCl_2$ and $MgCl_2$ were added to 0.1 $mol{\cdot}l^{-1}$ glucose-asparagine solutions in order to achieve salt concentrations ranging from 0.2 $mol{\cdot}l^{-1}$ to 6.0 $mol{\cdot}l^{-1}$. The volume of the model solutions was equal to 5 ml. The model solutions were heated in an oil thermostatic bath (Haake Phoenix, Thermo Electron, Karlsruhe, Germany) at 150 °C for 50 min. After heating, the samples were immediately cooled down under running cold water.

Preparation of the short-dough biscuits

Short-dough biscuits were prepared according to the slightly modified formulation by Gallagher et al. [43]. The formulation consisted of flour (Despar, Casalecchio di Reno, Italy), shortening (Unigrà, Conselice, Italy), saccharose (Carlo Erba, Milano, Italy), deionized water, glucose (Carlo Erba), sodium chloride (Carlo Erba), baking powder (sodium hydrogen carbonate, disodium diphosphate, dried starch; Cameo, Desenzano del Garda, Italy) and asparagine (Sigma-Aldrich, Milano, Italy). Asparagine fortification was carried out in order to better appreciate the salts effect on acrylamide development. Tab. 1 shows the short-dough biscuit formulation. To this basic formulation, 0.5% flour weight of CH_3COOK, $CaCl_2$, $MgCl_2$ or a mixture of $CaCl_2$ and $MgCl_2$ (1:1, w/w)

Tab. 1. Short-dough biscuit formulation.

Ingredient	Content in flour [%]
Flour	100.0
Shortening	40.0
Saccharose	35.0
Deionized water	20.0
Glucose	5.0
Sodium chloride	0.7
Baking powder	0.5
Asparagine	0.1

were added. This salt concentration corresponded to 4.3×10^{-2}, 2.9×10^{-2}, 2.1×10^{-2} $mol \cdot l^{-1}$ respectively for CH_3COOK, $CaCl_2$ and $MgCl_2$. In the case of the mixture, $CaCl_2$ and $MgCl_2$ molar concentrations were 1.4×10^{-2} and 1.0×10^{-2}, respectively. After mixing and a 30 min resting time at 4 °C, the dough was sheeted to 0.3 cm thickness, cut to a diameter of 7 cm and baked in an air-circulating oven (Salvis Thermocenter, Oakton, Vernon Hills, Illinois, USA) at 170 °C up to a final moisture of 2%. One batch of each sample was prepared.

Analysis of acrylamide

Acrylamide determination was carried out according to the method of ANESE et al. [44]. Briefly, 1 000 μl of an aqueous solution of 2,3,3[$^2H_3$] acrylamide (d_3-acrylamide) (0.20 $\mu g \cdot ml^{-1}$; Isotec, Sigma-Aldrich, Milano, Italy) as internal standard and 15 ml of MilliQ water (Millipore, Vimodrone, Italy) were added to 1 g of finely ground biscuit weighed into a 100 ml centrifuge tube. After extraction at 60 °C for 30 min under magnetic stirring, the mixture was centrifuged at 12 000 × *g* for 15 min at 4 °C (Avanti Centrifuge J-25, Beckman, Palo Alto, California, USA). Analogously, 1 000 μl of the internal standard (0.50 $\mu g \cdot ml^{-1}$) were added to 1 g of glucose-asparagine solution and diluted to 10 ml with MilliQ water. Aliquots of 10 ml of the clarified aqueous extract or of the glucose-asparagine solution with added d_3-acrylamide were cleaned-up by solid phase extraction (SPE) on an Isolute Env+, 1 g (Biotage, Uppsala, Sweden). The volume of the eluted fraction was reduced under vacuum to about 1.5–2 ml by using a rotary evaporator at a temperature of 80 °C, and filtered through a 0.45 μm membrane filter before the HPLC-MS analysis. LC-ESI-MS-MS in positive ion mode analyses were performed by a Finnigan LXQ linear trap mass spectrometer (Thermo Electron Corporation, San José, California, USA) coupled to a Finnigan Surveyor LC Pump Plus (Thermo Electron) equipped with a thermostated autosampler and a thermostated column oven. The analytical column was a Waters Spherisorb ODS2 (Waters Corporation, Milford, Massachusetts, USA, 250 × 2.0 mm, 5 μm). Elution was carried out at a flow rate of 0.1 $ml \cdot min^{-1}$, in isocratic conditions, at 30 °C using as mobile phase a mixture of 98.9% water, 1% methanol and 0.1% formic acid (v/v/v). Full scan MS/MS was carried out by selecting the ions at m/z 72 and m/z 75 as precursor ions for acrylamide and d_3-acrylamide, respectively. The area of the chromatographic peaks of the extracted ion at m/z 55, due to the transition 72 > 55, and at m/z 58, due to the transition 75 > 58, were used for the quantitative analysis. The quantitative analysis was carried out using the method of internal standard. The relative response factor of acrylamide with respect to d_3-acrylamide was calculated daily by analysing a standard solution. Limit of detection (*LOD*) was 25 $ng \cdot g^{-1}$, as derived from the calibration curve.

Analysis of 5-hydroxymethylfurfural (HMF)

5-Hydroxymethylfurfural was determined by HPLC according to the slightly modified method of GARCÍA-VILLANOVA et al. [45]. Briefly, 5 ml of Milli Q water were added to 1 g of ground biscuit into a 100 ml centrifuge tube. The sample was mixed with Polytron (Polytron PT-MR 3000, Kinematica, Littau, Switzerland) at 3 200 × *g* for 1 min and clarified with 0.5 ml each of Carrez I and Carrez II solutions. The resulting mixture was centrifuged at 9 500 × *g* for 15 min at 4 °C (Avanti Centrifuge J-25) and subsequently filtered through a 0.45 μm membrane filter before the HPLC analysis. HMF determination in glucose-asparagine model solutions was carried out by sample injection in HPLC without preliminary filtration. A HPLC system Varian Pro Star model 230 (Varian Associates, Walnut Creek, California, USA) equipped with a Varian Pro Star photodiode array detector (model 330, Varian Associates) was used. A Econosil C18 column (Alltech, Deerfield, Illinois, USA), 250 mm length, 4.6 mm internal diameter, 10 μm particle diameter was used. Injection volume was 20 μl and the mobile phase, delivered at a flow rate of 1 $ml \cdot min^{-1}$, consisted of 90% water and 10% methanol (Carlo Erba) in isocratic conditions. The detection wavelength was 280 nm. 5-Hydroxymethylfurfural (Sigma-Aldrich) was used as calibration standard. Peak integration was performed by the Software Chromatography Star IC 5.3 version (Varian Associates). *LOD* was 69 $ng \cdot g^{-1}$, as derived from the calibration curve.

Determination of water activity

Water activity (a_W) was determined by means of a dew-point measuring instrument (AQUA 17 LAB, Decagon, Pullman, Washington, USA) at 25 °C.

Determination of pH

The pH values were determined by using a pH-meter (Mettler Toledo 355, Lou Analyzer, Halstead Essex, United Kingdom) before and after the thermal treatment of the samples. In the case of biscuits, about 5 g of ground cooked sample were suspended in about 10 ml of sonicated deionized water.

Colour analysis

Colour analysis was carried out on finely ground biscuits by using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardized against a white tile before measurements. Colour was expressed in L^* (lightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness) scale parameters and a^* and b^* were used to compute the hue angle ($\tan^{-1} b^* a^{*-1}$), which is the attribute by which colour is identified as red, yellow, green and blue [46].

Determination of total solid content

Total solid content was determined by gravimetric method by drying the samples under vacuum (1.32 kPa) to constant weight, according to Association of Official Agricultural Chemists [47]. With respect to the official method, drying was carried out at 75 °C instead of 100 °C to avoid losses due to non-enzymatic browning and pyrolysis reactions.

Statistical analysis

The results reported are the average of three measurements. Coefficients of variation, expressed as the percentage ratio between the standard deviations and the mean values, were lower than 10 and 8 for acrylamide and HMF analyses, 9 for pH determinations, 2 for colour and a_W measurements, and 4 for total solid content determinations.

One-way analysis of variance was carried out and differences among means were assessed by using the Tukey test (Statistica for Windows, 5.1, Statsoft, Tulsa, Oklahoma, USA). Means were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

The effect of salts on acrylamide and HMF formation in model solutions

The effect of CH_3COOK, $CaCl_2$ and $MgCl_2$ on acrylamide and HMF development was first studied on glucose-asparagine model solutions, because these simple model systems are more reproducible and helpful for interpreting results ob-

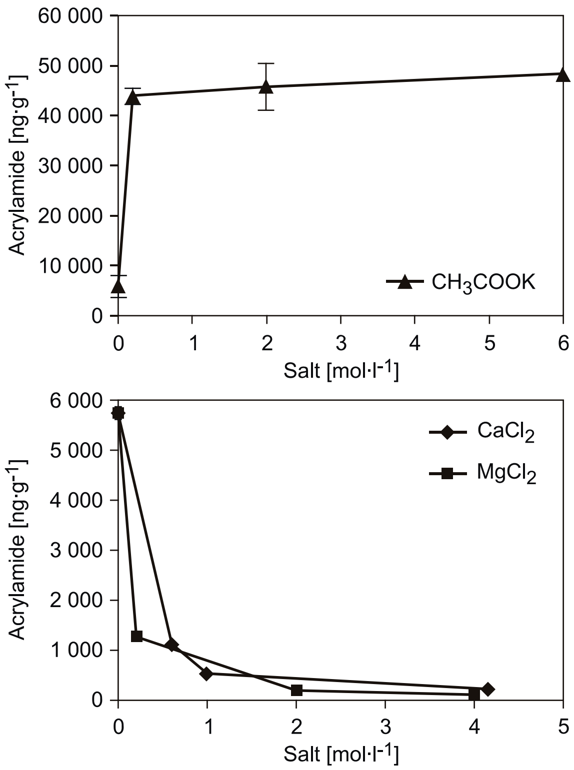

Fig. 1. Acrylamide concentration in glucose-asparagine model solutions as a function of CH_3COOK, and $CaCl_2$ and $MgCl_2$ concentrations.

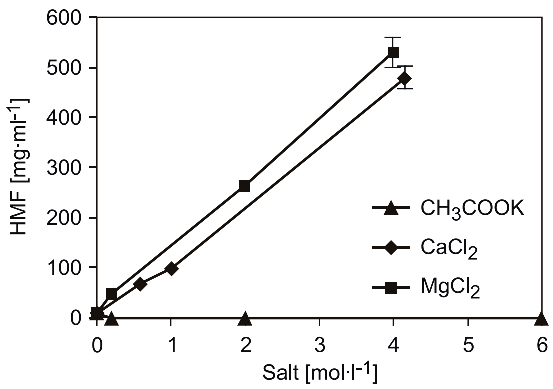

Fig. 2. HMF concentration in glucose-asparagine model solutions as a function of CH_3COOK, $CaCl_2$ and $MgCl_2$ concentrations.

Tab. 2. Water activity (a_w) and pH before and after heating of glucose-asparagine model solutions as a function of CH_3COOK, $CaCl_2$ and $MgCl_2$ concentrations.

Salt	Concentration [mol·l⁻¹]	a_w	pH before heating	pH after heating
CH_3COOK	0.0	0.98 ± 0.01	6.7 ± 0.0	5.7 ± 0.7
	0.2	0.99 ± 0.02	6.9 ± 0.2	5.4 ± 0.0
	2.0	0.91 ± 0.01	7.8 ± 0.3	6.3 ± 0.0
	6.0	0.73 ± 0.03	8.6 ± 0.2	7.3 ± 0.0
$CaCl_2$	0.0	0.97 ± 0.01	6.7 ± 0.0	5.7 ± 0.7
	0.6	0.96 ± 0.00	5.1 ± 0.2	4.6 ± 0.1
	1.0	0.93 ± 0.00	5.4 ± 0.5	4.2 ± 0.1
	4.2	0.72 ± 0.02	4.1 ± 0.2	2.8 ± 0.0
$MgCl_2$	0.0	0.96 ± 0.02	6.7 ± 0.0	5.7 ± 0.7
	0.2	0.98 ± 0.01	5.3 ± 0.0	5.6 ± 0.0
	2.0	0.89 ± 0.01	4.4 ± 0.3	4.0 ± 0.0
	4.0	0.72 ± 0.00	3.8 ± 0.2	3.0 ± 0.0

a_w and pH data are presented as mean values of triplicates ± standard deviation.

tained from real food products [48]. Fig. 1 shows the changes in acrylamide concentration of the glucose-asparagine model solutions as a function of CH_3COOK, $CaCl_2$ or $MgCl_2$ concentrations. It is possible to observe that, even at very low concentrations, CH_3COOK was responsible for a dramatic increase in acrylamide, whereas, depending on salt concentration, $CaCl_2$ and $MgCl_2$ decreased or even inhibited its formation. The latter results are in agreement with reports from the literature [19, 20, 23, 29]. On the contrary, no data are available on the influence of CH_3COOK on acrylamide formation. Opposite results were found for the HMF formation in the glucose-asparagine model solutions with added CH_3COOK, $CaCl_2$ or $MgCl_2$ (Fig. 2). In fact, the HMF concentration increased with the increase in $CaCl_2$ and $MgCl_2$ concentrations, while the addition of CH_3COOK had an inhibitory effect on the formation of the toxic molecule.

Since addition of salts may change the a_w and pH of the system, which in turn may affect acrylamide and HMF formation [36, 40, 49, 50], the influence of CH_3COOK, $CaCl_2$ or $MgCl_2$ on a_w and pH of the model solutions was evaluated (Tab. 2). In our experimental conditions, the addition of similar concentrations of the different salts caused only slight changes in a_w. Therefore, it is possible to assume that a_w exhibits a negligible role in the development of the heat-induced toxic molecules. The divalent cations Ca^{2+} and Mg^{2+} were capable of inducing significant pH reduction in the un-

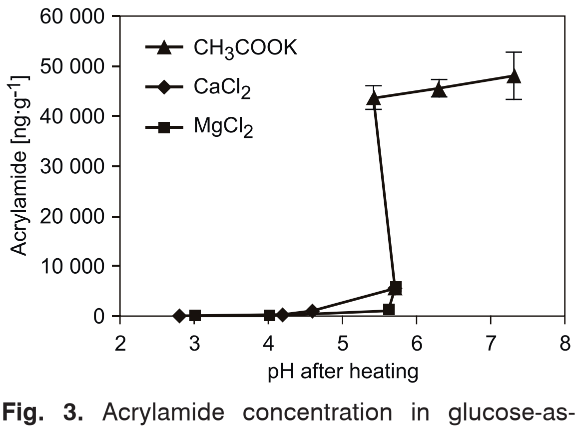

Fig. 3. Acrylamide concentration in glucose-asparagine model solutions added with CH_3COOK, $CaCl_2$ or $MgCl_2$ as a function of pH.

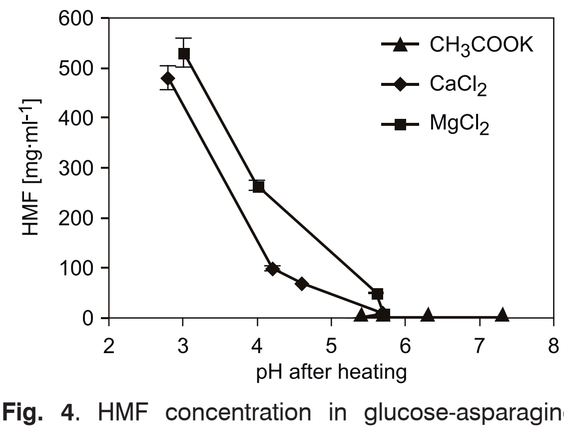

Fig. 4. HMF concentration in glucose-asparagine model solutions added with CH_3COOK, $CaCl_2$ or $MgCl_2$ as a function of pH.

heated model solutions [29]. The higher the cation concentration, the greater the pH reduction. On the contrary, the pH increased with the increase in the CH_3COOK concentration. Although heating was responsible for a decrease in pH as a consequence of the development of Maillard reactions [51], the CH_3COOK containing solutions always had significantly higher pH values than those added with $CaCl_2$ or $MgCl_2$. As acrylamide increased with increasing the pH (Fig. 3), HMF formation was favoured by low pH values (Fig. 4). These pH changes would explain the acrylamide and HMF levels found in the model solutions. Moreover, in Figs 3 and 4 it is possible to observe that, at a same pH value, the salts affected acrylamide and mainly HMF formation in a different manner. This result would suggest that not only the pH, but also the salt nature may influence the development of the toxic molecules.

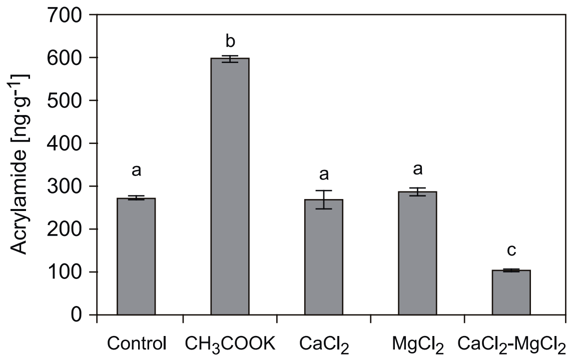

Fig. 5. Acrylamide concentration in control biscuit and in biscuits with added CH_3COOK, $CaCl_2$, $MgCl_2$ and $CaCl_2$-$MgCl_2$ mixture.
Different letters indicate a significant difference ($P < 0.05$) by Tukey test.

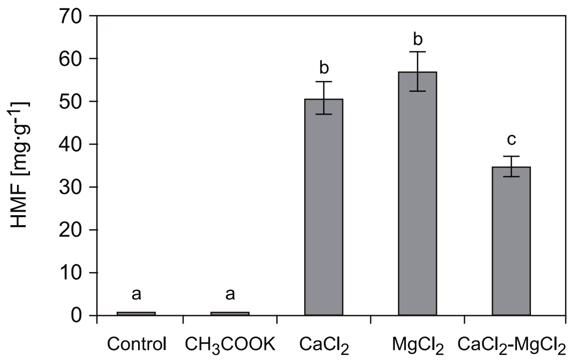

Fig. 6. HMF concentration in control biscuit and in biscuits with added CH_3COOK, $CaCl_2$, $MgCl_2$ and $CaCl_2$-$MgCl_2$ mixture.
Different letters indicate a significant difference ($P < 0.05$) by Tukey test.

The effect of salts on acrylamide and HMF formation in biscuits

The influence of 0.5% flour weight of CH_3COOK, $CaCl_2$, $MgCl_2$ and a $CaCl_2$-$MgCl_2$ mixture on acrylamide formation in short-dough biscuits was assessed. Preliminary trials showed that higher $CaCl_2$ and CH_3COOK concentrations were responsible for the generation of undesired off-flavours. Besides that, the salt concentrations used in this study were comparable with $CaCl_2$ concentrations considered by Levine and Ryan [29] in a wheat dough model system. Fig. 5 shows acrylamide concentrations in the control biscuit and in biscuits with added CH_3COOK, $CaCl_2$, $MgCl_2$ and $CaCl_2$-$MgCl_2$ mixture. As already observed for the model solutions, also in this case the addition of CH_3COOK considerably favoured acrylamide development. As compared to the control sample, the acrylamide increase was equal to 116%. On the contrary, biscuits with added $CaCl_2$ or $MgCl_2$ in the recipe presented acrylamide contents similar to the control sample ($P > 0.05$). This result is different from that observed for the model solutions. The incapability of $CaCl_2$ and $MgCl_2$ to minimize acrylamide formation in the biscuits may be due to the low salt concentration used as compared to that of the model solutions or, alternatively, to the complexity of the matrix where possible interactions among the salts and other components may occur. By contrast, a 60% reduction in acrylamide was obtained when $CaCl_2$ and $MgCl_2$ were added in combination to the biscuit dough, suggesting a synergic effect. As observed for the model solutions, also in this case the influence of salts on HMF development was opposite with respect to that on acrylamide formation (Fig. 6). In fact, the addition of CH_3COOK did not result in affecting the HMF formation, whereas the use of $CaCl_2$, $MgCl_2$ or their mixture greatly promoted the formation of the toxic molecule.

Tab. 3 shows the a_w and pH values of the doughs and biscuits with added CH_3COOK, $CaCl_2$, $MgCl_2$ and the $CaCl_2$-$MgCl_2$ mixture and of the corresponding control sample. In all cases, the biscuits with added salts in the recipe had comparable a_w and pH with those of the control before and after cooking. Therefore, it can be inferred that the development of the toxic molecules is mainly influenced by the salt nature as assumed for the model solutions. Similar conclusions were drawn by Levine and Ryan [29] for cooked wheat flour and water dough samples whose pH was adjusted to near 6 to compensate for the pH changes caused by the addition of Ca^{2+} ions. They found that the addition of 0.04 $mol \cdot l^{-1}$ $CaCl_2$ significantly reduced acrylamide formation by up to 36%.

Tab. 3. Water activity (a_w) and pH of doughs and biscuits added with CH_3COOK, $CaCl_2$, $MgCl_2$ and $CaCl_2$-$MgCl_2$ mixture.

Sample	Dough a_w	Biscuits a_w	Dough pH	Biscuits pH
Control	0.79 ± 0.01	0.19 ± 0.01	6.0 ± 0.5	6.1 ± 0.2
CH_3COOK	0.77 ± 0.02	0.19 ± 0.00	6.1 ± 0.2	6.4 ± 0.3
$CaCl_2$	0.78 ± 0.01	0.13 ± 0.00	6.0 ± 0.0	5.6 ± 0.5
$MgCl_2$	0.81 ± 0.00	0.20 ± 0.01	6.0 ± 0.3	5.8 ± 0.2
$CaCl_2$-$MgCl_2$	0.79 ± 0.05	0.15 ± 0.02	6.0 ± 0.3	6.1 ± 0.1

a_w and pH data are presented as mean values of triplicates ± standard deviation.

The biscuits were analysed also with regard to colour (Tab. 4). The biscuits with added $CaCl_2$ or $MgCl_2$ had L^* and hue angle values similar to those of the control sample. In addition, the $CaCl_2$-$MgCl_2$ mixture slightly but significantly increased the L^* value of the biscuits, while it did not affect the hue angle as compared to the control sample. On the contrary, the addition of CH_3COOK significantly reduced L^* and hue angle values ($P < 0.05$), indicating a more intense browning development, which was concomitant with the formation of high acrylamide concentrations. This observation is in agreement with literature reports [52].

Tab. 4. Lightness and hue angle of control biscuits and biscuits added with CH_3COOK, $CaCl_2$, $MgCl_2$ and $CaCl_2$-$MgCl_2$ mixture.

Salt	Lightness (L^*)	Hue angle ($\tan^{-1} b^*.a^{*-1}$)
Control	70.7 ± 1.3^a	63.1 ±0.2^a
CH_3COOK	64.8 ± 1.1^b	60.4 ±0.4^b
$CaCl_2$	69.0 ± 1.4^a	61.9 ±0.9^a
$MgCl_2$	70.4 ± 0.6^a	63.7 ±0.2^a
$CaCl_2$-$MgCl_2$	72.9 ± 0.4^c	63.3 ±0.7^a

Different letters indicate a significant difference ($P < 0.05$) by Tukey test.

CONCLUSIONS

The results of this study clearly show that salts capable of reducing acrylamide formation in both glucose-asparagine model solutions and biscuits were responsible for HMF increase. For instance, in our experimental conditions, a 60% acrylamide reduction in the biscuits was achieved by adding a 0.5% (flour weight) $CaCl_2$-$MgCl_2$ mixture, which however caused the formation of very high levels of HMF.

Although the addition of some cations (Ca^{2+}, Mg^{2+}) to certain bakery products has been suggested as an option to reduce acrylamide levels [30], our results raise a question regarding the addition of these salts in the recipe, due to their promoting effect on HMF development. Moreover, as is known, the use of these salts may favour the formation of other process contaminants, such as 3-monochloropropane-1,2-diol (3-MCPD) [53]. Therefore, a route that seems optimal for acrylamide reduction should be considered also for its potential unwanted side effects. In the light of these considerations, it would be better to choose alternative strategies to minimize acrylamide formation, which may not be problematic in terms of health risks.

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