Controlling the Maillard Reaction by Reactant Encapsulation: Sodium Chloride in Cookies

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Abstract: Formation of Maillard reaction products (MRPs) including 5-hydroxymethylfurfural (HMF) and acrylamide has been an intensive area of research in recent decades. The presence of reactants such as sodium chloride may influence the Maillard reaction (MR) pathways through the dehydration of various key intermediates. The aim of this work was to test the potential of ingredient encapsulation to mitigate the MR by investigating the case of sodium chloride encapsulation on the HMF formation in cookies. Thirteen cookies were prepared with recipes containing free or encapsulated NaCl. Increasing NaCl concentration from 0 to 0.65% increases HMF concentration up to 75%, whereas in the presence of encapsulated NaCl the reduction of HMF varied from 18 to 61% due to the inhibition of sucrose pyrolytic decomposition and the fructofuranosyl cation formation. Data demonstrated that the more heat-resistant the lipid-based coating was, the more pronounced the reduction of HMF formation. The results showed that encapsulation represents a useful approach to prevent the formation of potentially harmful compounds in thermally processed foods.

Keywords: encapsulation, HMF, Maillard reaction, NaCl

Introduction

In recent decades the Maillard reaction (MR), that is, the reaction between reducing sugars and amino acids, proteins, or simple amines, has been intensively studied. The MR pathways, which are fundamental in heated food, lead to the formation of many Maillard reaction products (MRPs): flavoring compounds, nonenzymatic browning products, high molecular weight compounds (melanoidins), reductones, amino-reductones, nonenzymatic browning products, high molecular weight compounds (melanoidins), reductones, amino-reductones. Some of these compounds gave desired features to the final products, but others are mutagenic, carcinogenic, or cytotoxic. 1–4

Acrylamide and 5-hydroxymethylfurfural (HMF) are potentially toxic compounds; in particular, acrylamide has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans, 5,6 although there are contradictory findings on the possible carcinogenicity of HMF. HMF in vitro genotoxicity was observed when a specific sulfotransferase catalyzes the formation of the reactive metabolite 5-sulfoxymethylfurfural. 7

Several mitigation strategies have been proposed to limit the formation of HMF and acrylamide. 8 The mitigation procedures addressed the process conditions and/or technologies, such as modifying time-temperature of frying or baking. Alternatively, reformulation, selections of cereal or potato varieties low in MRP precursors, and the addition of glycine, cysteine, natural antioxidant, or enzyme have been proposed as effective procedures in different products. 6,8

Since the discovery of acrylamide formation in heated food, the matter of the study has been increasingly deepened. 6,9 Bivalent and monovalent ions such as sodium chloride may influence MR development through the dehydration of various key intermediates. Gökmen and Şenyuva 10 demonstrated that the addition of polyvalent cations such as Ca 2+ prevents acrylamide formation in an asparagine-glucose model system, but at the same time the formation of HMF and furfurals was significantly increased. Levine et al. 11 found in a dough model system that acrylamide concentration decreased upon the increase of sodium chloride concentration. Carle et al. 12 showed that sodium chloride plays a dual role in acrylamide formation: at NaCl concentrations from 1 to 2% acrylamide formation decreases significantly; instead, increasing salt concentration more than 2% led to an increase of acrylamide content. Gökmen and Şenyuva 10 found in a liquid model system constituted by 10 μmol/L of asparagine and glucose that the final acrylamide concentration was higher for the control, declined for NaCl concentrations from 0.5 to 5 μmol/L, and significantly increased for NaCl concentrations from 5 to 20 μmol/L.

On the other hand, HMF formation is more clearly linked to NaCl concentration: monovalent cations favored the dehydration of key intermediates, leading to the increase of HMF formation. 10 This hypothesis is widely demonstrated both in commercial dextrose preparations 14 and in cookie model systems 17 in the presence of 0.45 and 0.7% of NaCl, respectively.

Ingredient encapsulation is characterized by the incorporation of bioactive molecules in small capsules that release their...
contents at controlled rates over prolonged periods of time. Encapsulation in foods has been already used to avoid the degradation of bioactive compounds: coating allowed the physicochemical protection of functional molecules and ingredients. Vitamins, antioxidant, flavorings, and preservatives have been incorporated using appropriate coatings according to the desired functionality.

Encapsulation can be a smart approach to limit the contact between reactants in food matrix, so it can be used to modulate the Maillard reaction during thermal treatment.

In this paper the use of encapsulation to prevent the formation of undesired MRPs was investigated. As the effect of salt in promoting HMF formation was well established in different systems, NaCl encapsulation was selected to verify the efficacy of encapsulation strategy to prevent HMF formation in foods. To this purpose differently coated NaCl preparations were added in the formulation of cookies, monitoring the formation of HMF and acrylamide at different cooking times. Results demonstrated that encapsulation of NaCl significantly decreased HMF formation without affecting the color or sensorial properties of the cookies.

**MATERIALS AND METHODS**

**Chemicals.** Acetonitrile, water, and methanol for HPLC and LC-MS/MS determination and sodium chloride were obtained from Merck (Darmstadt, Germany). Formic acid (98%) was purchased from J. T. Baker (Deventer, The Netherlands). Acrylamide, [2,3,3-d4]-acrylamide, 5-hydroxymethylfurural (HMF) standard, and sucrose were purchased from Sigma (St. Louis, MO, USA). All of the samples were filtered through 25 mm diameter and 0.45 μm pore size nylon filter using a 2.5 mL conventional syringe (BD, Franklin Lakes, NJ) equipped with a PTFE adapter (Phenomenex, Torrance, CA, USA). Carrez reagent potassium salt and Carrez reagent zinc salt were purchased from Sigma (St. Louis, MO, USA). All of the samples were filtered through 25 mm diameter and 0.45 μm pore size nylon filter using a 2.5 mL conventional syringe (BD, Franklin Lakes, NJ) equipped with a PTFE adapter (Phenomenex, Torrance, CA, USA). Carrez reagent potassium salt and Carrez reagent zinc salt were purchased from Carlo Erba (Milano, Italy).

**Encapsulation Process.** Microencapsulation by spray-coating was performed using a UNI-GLATT device in a bottom spray configuration (Glatt, Germany). Sodium chloride (Labogros, Saint Herblain, France) was coated with three different materials: a melted fatty acid blend (stearic acid TP 18/55, MP = 55 °C), candelilla wax (mp = 70 °C), and carnauba wax (mp = 85 °C). All of the materials were purchased from Interchimie (Bobigny, France). Spray-coating parameters were adjusted to obtain a homogeneous coating. The coating material was sprayed through a nozzle onto fluidized sodium chloride. Droplets progressively covered the sodium chloride surface to achieve a uniform coating after a few minutes of operation.

Blank microparticles were produced by spray-cooling. The material (stearic acid TP 18/55, candelilla wax, or carnauba wax, respectively) was melted and heated above the melting temperature. Melted material was distributed on a spinning disk by a peristaltic pump. Microdroplets were ejected by centrifugal force and solidified by air-cooling (room temperature) to produce solid microparticles.

**Melting Point Measurement.** The melting points of encapsulated salt and its respective empty coating material were measured using a TA Q20 model DSC apparatus (TA Instruments, New Castle, DE, USA). The apparatus was calibrated with indium (mp = 156.6 °C, ΔH = 28.5 J/g). The DSC runs were operated under nitrogen gas atmosphere (30 mL/min) using an empty pan as the reference. After approximately 2 mg of material was weighed, the aluminum pan was hermetically sealed. The pan was placed into the apparatus and scanned over an appropriate temperature range at a heating rate of 5 °C/min.

**Cookie Samples.** Model cookies were prepared according to a recipe described in AACC (American Association of Cereal Chemists) method 10-54 (AACC, 2000) with some modifications. Refined flour and shortening were kindly supplied by Kraft Foods (Glattpark, Switzerland), whereas ammonium bicarbonate and sodium bicarbon-

<table>
<thead>
<tr>
<th>Recipe</th>
<th>NaCl coated NaCl microparticles</th>
<th>NaCl coated materials microparticles</th>
</tr>
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<tbody>
<tr>
<td>NaCl 0%</td>
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<td>0</td>
</tr>
<tr>
<td>NaCl 0.32%</td>
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<td>0.100</td>
</tr>
<tr>
<td>NaCl 0.65%</td>
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<td>0.100</td>
</tr>
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<td>NaCl 1%</td>
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<td>0.100</td>
</tr>
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<td>0.100</td>
</tr>
<tr>
<td>(SPAB) microparticles + NaCl</td>
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<td>0.100</td>
</tr>
<tr>
<td>CanW</td>
<td>0.610</td>
<td>0.110</td>
</tr>
<tr>
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<td>0.110</td>
</tr>
<tr>
<td>CarW</td>
<td>0.625</td>
<td>0.125</td>
</tr>
<tr>
<td>(CarW) microparticles + NaCl</td>
<td>0.500</td>
<td>0.125</td>
</tr>
</tbody>
</table>

“HMF formation in foods. To this purpose differently coated NaCl preparations were added in the formulation of cookies, monitoring the formation of HMF and acrylamide at different cooking times. Results demonstrated that encapsulation of NaCl significantly decreased HMF formation without affecting the color or sensorial properties of the cookies.

**Preparation of Model Systems.** A model system composed of sucrose and NaCl was used to determine the effect of salt on HMF formation. A total of 10 μmol of sucrose and NaCl was transferred to a 25 mL test tube (Pyrex, 25 mL) as their aqueous solution. Total reaction volume was adjusted to 100 μL with deionized water. A total of 300 mg of silica gel was added to cover the reaction mixture, and the tube was tightly closed with a screw cap. The reactions were performed in an oil bath at 200 °C for 5, 10, and 20 min. All reactions were performed in triplicate. The reaction mixtures after heating were suspended in 2 mL of 10 mM formic acid, and the aqueous extract was obtained by vortexing the tube for 2 min. After centrifugation at 11800 g for 5 min, 1 mL of the supernatant was passed through a 0.45 μm nylon syringe filter into a vial.

**Physical Analysis of Microparticles.** Particle Size Distribution. Microparticle size distributions were determined by laser granulometry (Malvern Mastersizer, Malvern, UK). Samples were analyzed in triplicate, 5000 particles were counted for each analysis.

**Optical Microscopy.** Microparticle pictures were obtained with a motic binocular microscope (magnification x30) equipped with an SMZ motic camera (motic, Germany).

**Scanning Electron Microscopy (SEM).** The microparticle morphology was observed by SEM (JSM 5800, JEOL, Japan). The microparticles were mounted on a support platform (9 mm diameter) with a conductor adhesive (carbon tabs, Agar Scientific) and fixed with a thin carbon layer (ca. 20 nm). To make the material conducting, the
sample was covered with a fine layer of gold (step of metalization). The pressure was set to 10⁻³ mPa during the metalization and to 6 × 10⁻⁶ mPa inside the apparatus.

**Conductimetry.** Conductivity experiments were carried out using two different devices. Sodium chloride content was measured using a WTW 315i conductimeter equipped with a TetraCon 325 probe (WTW, Germany). Microparticle samples were dispersed in water and crushed prior to titration. All measurements were performed in triplicate. Sodium chloride cumulative release in water was followed with a T50 titrator equipped with an InLab 730 probe and an internal dilution system (WTW, Germany). Microparticle samples were dispersed in water and the resulting mixture was centrifuged at 10,000 g for 10 min at 4 °C. The supernatant was then collected in a 20 mL volumetric flask, and two further extractions were performed using 5 mL of deionized water. Finally, the pellets were discarded, and the supernatants were filtered through a 0.45 μm nylon filter; 2 mL was collected and used for HPLC analysis. The HPLC system consisted of LC-10AD class VP pumps and an SPD-M10A diode array detector equipped with an SCL-10A class VP controller, all from Shimadzu (Kyoto, Japan). The mobile phase was a mixture of acetonitrile in water (5% v/v) at a flow rate of 12 L/h, and the gradient elution was applied: 0–3 min, 100% A; 3–8 min, 93% A; 8–12 min, 100%, at a flow rate of 0.800 μL/min, whereof 200 μL was split into the ion source.

The quantification was carried out in multiple reaction monitoring (MRM) at m/z ratios of 72 and 75 for acrylamide and [2,3,3-d₃]-acrylamide, respectively. Specific molecular fragments corresponding to m/z SS and 44 and m/z 58 and 44 were also monitored. The daughter ions were obtained through fragmentations with the following specific conditions: the source temperature was set at 350 °C, nitrogen was used as nebulizer gas at a flow rate of 12 L/h, and the needle and cone voltages were set at 3.0 kV and 100 V, respectively. Under the above-mentioned chromatographic conditions the acrylamide and its corresponding isotope standard eluted at 7.4 min. Acrylamide was quantified using a linear calibration curve built with specific solutions of acrylamide and [2,3,3-d₃]-acrylamide dissolved in water (50–500 ng/mL). The LOD and LOQ were, respectively, 10 and 30 ng/mL for acrylamide, and the coefficient of determination r² was 0.9998. The internal standard was used for the recovery test, and it varied from 95 to 103%. All of the analyses were performed in quadruplicate and the results expressed as nanograms per gram of sample.

**High-Resolution Mass Spectrometry Analysis (HRMS) of Reaction Products Formed in the Model System.** Extracts of model systems were analyzed by HRMS to identify the reaction intermediates and products. A Thermo Scientific Accela UHPLC system (San Jose, CA, USA) coupled to a Thermo Scientific Exactive Orbitrap HRMS was used. The HRMS system was operated in positive electrospray ionization mode. The chromatographic separations were performed on an Atlantis T3 column (250 mm × 4.6 mm i.d., 5 cm) (Waters Corp, Milford, MA, USA) using 0.05% aqueous formic acid and methanol isocratically (70:30) at a flow rate of 0.5 μL/min (30 °C) for 15 min. The scan analyses were performed in an m/z range between 50 and 600 at ultrahigh resolving power (R = 100 000). The data acquisition rate, the automatic gain control target,
and maximum injection time were set to 1 Hz, 1 × 10⁶, and 100 ms, respectively. The source parameters were as follows: sheath gas flow rate, 45 (arbitrary units); auxiliary gas flow rate, 20 (arbitrary units); sweep gas flow, 3 (arbitrary units); spray voltage, 3 kV; capillary temperature, 300 °C; capillary voltage, 25 V; tube lens voltage, 55 V; and vaporizer temperature, 300 °C. To confirm the reaction path leading to HMF, possible forms of sucrose decomposition products were extracted from the total ion chromatograms.

**Sensory Analysis.** To determine if there was a detectable difference among the cookies with different recipes, a triangle test was undertaken according to UNI ISO 4120 Norm. Two sessions of 30 untrained panelists were conducted at 20–22 °C in an eight booth sensory panel room equipped with white fluorescent lighting. The various samples were put in different vessels that were coded uniformly, using three-digit numbers chosen at random for each test to prevent stimulus errors, logic errors, or suggestion effect. In each session, panelists were asked to complete the test on two types of cookies consisting of two samples with 0.65% of sodium chloride and one at the same encapsulated sodium chloride concentration, as odd samples. The panelists were asked to taste the samples and decide which sample was different from the others; in the first session they evaluated the general differences, and in the second session they were asked to decide the differences in salty taste. For the two sessions a forced-choice procedure was used. The level of significance was α = 0.01. It was decided to evaluate if any difference was detectable in the samples.

**Statistical Analysis.** Data were analyzed by ANOVA, and means were compared by Duncan’s multiple-range test.

### RESULTS AND DISCUSSION

Sodium chloride (mean diameter = 556 μm) was successfully encapsulated using the fluidized-air bed coating. Microparticles with mean diameters of 722, 720, and 716 μm were obtained for stearic/palmitic acid blend (SPAB), candelilla wax (CanW), and carnauba wax (CarW) coatings, respectively. Sodium chloride content was measured by conductimetry after crushing and total release in water and was titrated at 877 ± 8, 752 ± 10, and 774 ± 13 mg/g of microparticles (for SPAB, CanW, and CarW coatings, respectively). As shown in Figure 1 (top panels), encapsulated sodium chloride microparticles present intermediate shapes from cubic (sodium chloride typical shape, thin coating) to spherical (thick coating). Only a few microparticles present poor or the absence of coating. Microparticle surface morphology was analyzed by SEM. SEM images highlight the differences of the surface roughness: particles coated with CarW revealed a very smooth surface compared to those coated with CanW or SPAB.

The barrier ability of the coating to isolate sodium chloride from the external environment was first evaluated by conductimetry. Microparticles were dispersed in water, and the release of sodium chloride was monitored over time by plotting the increase of water conductivity. Blank microparticles made of coating material showed no significant modification of water conductivity (data not shown). Results with coated sodium chloride microparticles shown in Figure 2 demonstrate that the carnauba coating had the slowest release compared to candelilla wax coating or stearic/palmitic acid blend coating.

The differences among CarW, CanW, and SPAB coatings were due to the homogeneity of the coating. The more homogeneous the coating was, the less the sodium chloride was released. Roughness and heterogeneous coating structure that appeared on SEM pictures could allow a better water access inside the microparticles and a faster release of sodium chloride. NaCl encapsulation was already reported in the literature mainly to verify the release of the oil electrolytes in water.

Emulsion systems, however, its use to modulate the development of chemical reactions in foods has never been proposed thus far. In this case the slow melting of coating during cookie coating might reduce the participation of NaCl to chemical reactions and, at the same time, should guarantee its presence at the end of baking.

In Figure 3 the effect of NaCl concentration on the formation of HMF and acrylamide in cookies was reported.

Data clearly showed that NaCl promoted the formation of HMF in cookies. This result confirmed those previously obtained in model systems and for the first time demonstrated that the presence of 0.65% NaCl, which is the standard concentration of salt used in many commercial cookies, increased the formation of HMF up to 75%. NaCl showed a specific effect toward HMF, whereas the formation of acrylamide was not significantly modified by the presence of salt. Cookies with 0.65% (0.109 mol/kg) of NaCl showed an average acrylamide concentration of 0.278 ± 0.040 μg g⁻¹, whereas the control without NaCl had the highest concentration: 0.313 ± 0.057 μg g⁻¹. These data confirmed previous studies already mentioned in the Introduction showing that there is not a direct relationship between NaCl concentration and acrylamide formation.
The mechanisms leading to conversion of sucrose into HMF through the fructofuranosyl cation at high temperatures have been previously described.23 As shown in Figure 4, both glucose and fructofuranosyl cation can generate HMF by the elimination of 2 and 3 moles of water, respectively. The model sucrose system heated at 200 °C formed HMF with an initial rate of 1.11 nmol min⁻¹. With NaCl, the rate of HMF formation from sucrose increased to 8.13 nmol min⁻¹ (Figure 5). This confirmed the catalytic role of sodium in the pyrolysis of sucrose leading to HMF. It was a fact that the presence of NaCl accelerated the pyrolytic decomposition of sucrose during heating at 200 °C. The rate of sucrose decomposition increased from 2.85 to 10.18 μmol min⁻¹ when NaCl was present in the reaction mixture during heating. It is thought that NaCl as a metal cation acts as a Lewis acid in the reaction mixture that accelerates the decomposition of sucrose. It has been previously shown that dehydration of hexoses is catalyzed by organic acids, inorganic acids, salts, and Lewis acids.14,23

Formation of key intermediates in the heated model reaction mixtures was monitored to understand better the role of NaCl in sucrose decomposition in a semiquantitative way. Scan HRMS analyses of sucrose pyrolysates with and without NaCl tentatively confirmed the presence of 3-deoxyglucosone and 3,4-dideoxyosone, together with HMF having m/z of 163.0601, 145.0495, and 127.0390, respectively, with a very high mass accuracy (Δ < 2.0 ppm). Extracted ion chromatograms of these compounds in the pyrolysate of sucrose heated with NaCl at 200 °C for 10 min are shown in Figure 6. The rates of the formation of 3-deoxyglucosone and 3,4-dideoxyosone from sucrose increased by factors of 4.3 and 23.5 times in the presence of NaCl during heating as shown in Figure 7.

To verify the effectiveness of the encapsulation strategy to mitigate the formation of potentially harmful compounds, the HMF was an appropriate target and the NaCl a suitable reagent to be modulated by encapsulation. To prevent NaCl
participation to the dehydration of key intermediates for HMF formation, NaCl should be coated using a material that melts near the end of the cookies’ baking time. In fact, at the end of the baking time NaCl should be free because of the sensorial need to have some salty sensation in the cookies.

The effect of using NaCl encapsulated ingredients on HMF formation is shown in Figure 8. Using three different coating materials the encapsulation of NaCl led in all cases to a significant inhibition of HMF formation. Carnauba wax proved to be the most effective coating, giving an HMF concentration comparable to that of cookies without NaCl. In particular, HMF reductions were 18, 41, and 61% for the stearic/palmitic acid blend, candelilla wax, and carnauba wax coatings, respectively.

The results of this experiment fully confirmed the correctness of the proposed strategy: subtracting the reaction an agent catalyzing the HMF formation is an effective mitigation strategy to prevent its formation, and in this respect encapsulation proved to be a very effective tool.

Analysis revealed that the heat resistance of the coating is inversely correlated with the HMF formation, thus confirming that coating melting point was a key factor to determine the final HMF concentration. Blocking NaCl inside the microparticles reduces the time of its participation to the reaction converting sucrose into HMF. The increase of the melting point of the coating delays sodium chloride release and reaction during the oven baking. With regard to the baking of the cookies, the coating quality plays an important role, too. A homogeneous coating decreases water access to sodium and, finally, sodium chloride release and reaction. The melting point of the coating is inversely correlated with the amount of HMF formed in cookies during baking (y = −3.3119x + 358.87) with a correlation coefficient of 0.931. Obviously, encapsulating material with a higher melting point kept NaCl inside the microparticles, preventing its catalytic action on the formation of HMF from sucrose.

The baking conditions determine the complete melting of microparticles coating at the end of the baking time. This was confirmed by the sensory analysis results summarized in Table 2. A panel of untrained consumers was not able to distinguish the cookies manufactured with free NaCl from those obtained with encapsulated salt. In fact, a triangle test failed to reach the threshold of minimum correct answer; therefore, the sensory study confirmed that that encapsulated sodium chloride recipes were not perceived as different from that containing free 0.65% sodium chloride.

![Figure 7. Amounts of (a) 3-deoxyglucosone and (b) 3,4-dideoxyosone formed during heating of sucrose with and without NaCl at different time points.](image)

![Figure 8. HMF concentration in cookies prepared with different types of encapsulated NaCl. All samples have the same amount of NaCl (0.5 g). SPAB, encapsulated NaCl with stearic/palmitic acid blend coating; CanW, encapsulated NaCl with candelilla wax coating; CarW, encapsulated NaCl with carnauba wax coating. Significant differences among HMF contents were determined by ANOVA analysis and Duncan’s multiple-range test (p ≤ 0.05).](image)

### Table 2. Triangle Test Results on Cookies

<table>
<thead>
<tr>
<th>cookies served as odd sample</th>
<th>no. of assessors</th>
<th>no. of correct/incorrect judgments</th>
<th>minimum correct answer</th>
</tr>
</thead>
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<td>(A) Number of Correct/Incorrect Responses for the General Differences</td>
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<tr>
<td>encapsulated NaCl</td>
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<td>12/27</td>
<td>17*</td>
</tr>
<tr>
<td>CanW</td>
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<tr>
<td>encapsulated NaCl</td>
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<td>11/24</td>
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<tr>
<td>CarW</td>
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<tr>
<td>encapsulated NaCl</td>
<td>30</td>
<td>9/28</td>
<td>17*</td>
</tr>
<tr>
<td>SPAB</td>
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<tr>
<td>(B) Number of Correct/Incorrect Responses for Salty Taste</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>encapsulated NaCl</td>
<td>30</td>
<td>9/26</td>
<td>17*</td>
</tr>
<tr>
<td>CanW</td>
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<tr>
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<td>SPAB</td>
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</table>

*The panelists were asked to identify any differences between the two samples; α was set at 0.01. The minimum number of correct responses needed to conclude that two samples are similar, based on a triangle test, was 17; *, significant. SPAB, stearic/palmitic acid blend; CanW, candelilla wax; CarW, carnauba wax.
In conclusion, this is the first paper demonstrating that the encapsulation of some ingredients can be used to prevent the formation of undesired MRP in thermally treated foods.

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**Notes**

The authors declare no competing financial interest.

**Abbreviations Used**

MRPs, Maillard reaction products; HMF, 5-hydroxymethylfurural; HRMS, high-resolution mass spectrometry; SPAB, stearic palmitic acid blend; CanW, candellilla wax; CarW, carnauba wax.

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