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Investigation of the Correlation of the Acrylamide Content and the Antioxidant Activity of Model Cookies

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The purpose of this study was to evaluate the correlation between the acrylamide (AA) content and the antioxidant activity (AOA) of self-prepared cookies. Cookies were baked in the laboratory under defined conditions following four different recipes. The parameters of investigation were the influence of the type and relative content of sugar (glucose and fructose) and the baking time on the AA content as well as AOA of the final products. Parameters depending on the recipe and baking conditions such as the moisture content, the total nitrogen concentration, and the color of the products were evaluated for all samples as well. To prove the transferability of the findings gained with model cookies to samples from industry, the same measurements were performed on seven different types/brands of cookies that were purchased in local markets. A direct correlation was found between the concentration of AA and the AOA. With increasing baking time, the moisture content of the cookies decreased. The latter parameter correlated well with the AA concentration and AOA. The use of fructose enhanced the concentration of AA and the AOA of the final products, when compared with the use of sucrose. However, a simple model for the prediction of acrylamide contents and the AOA of samples from the baking time, color, protein, or moisture content of the samples was not found.

KEYWORDS: Acrylamide; antioxidant activity; Maillard reaction; cookies

INTRODUCTION

For almost 3 years now, a significant amount of research activities have focused on the mitigation of the formation of the probable carcinogen, acrylamide (AA), during the processing of starch-rich food (*1*, *2*). In an effort to reduce the amount of acrylamide formed, several strategies were proposed such as the reduction of the processing temperature, an earlier endpoint of processing, and/or the modification of recipes (*3*, *4*). The described examples lack by far completeness. About 15 patent applications concerning the reduction of AA formation during processing of food have been registered until now at the U.S. patent office (*5*). The food industry is also seeking solutions to minimize AA generation. Recently, the so-called toolbox concept was presented by the Confederation of the Food and Drink Industries of the EU (CIAA), which provides different measures for the reduction of the AA content, each more or less applicable depending on the food commodity (*6*).

However, there is broad agreement that AA is preferably formed from asparagine and reducing sugars through the Maillard reaction (*7*−*10*), the latter also being responsible for the generation of the brown color and many desirable sensorial active components of food (*11*). Concerning the toxicity of Maillard reaction products (MRPs) other than AA, many controversial findings were reported. Some MRPs were associated with a variety of diseases such as diabetes and cancer (*12*). It has been hypothesized that carcinogenic and mutagenic heterocyclic amines and β-carboline derivatives are formed in the Maillard reaction as well (*13*). Substances with 3(2*H*)-furanone structures have been reported as also having DNA-damaging properties (*14*, *15*). In 2004, the U.S. Food and Drug Administration (FDA) published the findings of high levels of carcinogenic furan in processed food, which is also generated during the Maillard reaction (*16*).

However, on the basis of published risk/exposure data for foodstuffs and environmental hazards, the particular hazard of human carcinogens originating as a result of MRPs seems to be relatively small (*17*). In contrast, many positive effects have been attributed to MRPs, such as antioxidant activity (AOA), which was first observed in the early 1950s (*18*). The exact nature of the antioxidants formed is not yet well-known. They are composed of high molecular weight substances without defined structures, such as melanoidins (*19*, *20*). Pronyl-lysine, a MRP, present in bread crust has demonstrated to have beneficial effects on human health (*21*). Similar results have been published referring to the AOA of coffee (*22*, *23*) and malt products (*24*). The AOA of MRPs also contributes substantially to the shelf life of heat-treated food (*25*). A detailed review on toxicology and antioxidant activities of nonenzymatic

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browning reaction products has been published by Lee and Shibamoto (*26*). However, until now there is not any conclusive study that put beneficial and undesirable properties of Maillard reaction products side by side and that quantifies an overall threat or benefit.

The major concern of food producers in taking actions to reduce the AA content of food was that the flavors and textures of food might be unacceptably changed by the applied strategy, having finally economic impacts. Concerning the health impact of adjusted process conditions, it was several times highlighted that the reduction of the frying temperature of potato products would lead to a higher fat content of the products, which is of course contradictory to all attempts to reduce obesity. But apart from that, there is very little information about the correlation between mitigation of acrylamide and its influence on beneficial properties of food, such as the AOA. Measures to reduce the content of one particular component could at the same time lead to the loss of many beneficial properties/components.

The objective of this study was to make a step toward a more holistic view on food by investigating the relationship between the AA content and the AOA of model cookies. These model cookies were self-prepared and were baked under different controlled conditions. The study focused on the influence of the composition of the pastries and the effect of baking conditions on the final product. In varying these parameters more than 50 different cookies were produced, knowing that many of them were just suitable for analytical purposes and would never have been consumed as such, e.g., due to very low or very high degree of browning. However, such big variations of the recipe and the processing conditions were necessary in order to identify interconnections. Sensorial properties of the products or edibility were in that respect of secondary importance and were therefore not considered in this study. The parameters that were measured, besides the AA content of the products, were their antioxidant activity (AOA), their water content, their total nitrogen content, and the color of the cookies.

MATERIALS AND METHODS

Preparation of Model Cookies. The model cookies were prepared according to four different recipes. All ingredients were purchased in local supermarkets. Percentages of sucrose/fructose content are rounded to whole numbers:

Recipe 1: 190 g of wheat flour (type 45), 250 g of cream, 3 eggs (about 150 g), 1 spoon (about 10 g) of baking powder, and 135 g of sucrose, the latter corresponding to about 21% w/w of the pastry.

Recipe 2: 500 g of wheat flour (type 45), 100 g of butter, 5 eggs (about 250 g), and either 0, 125, 250, 375, or 500 g of sucrose, the latter corresponding to 0, 13, 23, 31, or 37% w/w of the pastry, respectively.

Recipe 3: 500 g of wheat flour (type 45), 250 g of butter, 5 eggs (250 g), 1 package (20 g) of baking powder, and either 0, 100, 200, or 400 g of sucrose, the latter corresponding to 0, 9, 16, or 28% w/w of the pastry, respectively.

Recipe 4: 500 g of wheat flour (type 45), 250 g of butter, 5 eggs (250 g), 1 package (20 g) of baking powder, and either 100, 200, or 400 g of fructose, the latter corresponding to 9, 16, or 28% w/w of the pastry, respectively.

The ingredients were thoroughly mixed according to the recipe. Portions of 200 g of the pastry were rolled out to disks of 23 cm internal diameter after which trays of these disks were then baked in a household oven at 180 °C. Four dough disks were produced from each recipe and baked at different baking times, thus resulting in four different cookies. The final products were then ground and homogenized, all under liquid nitrogen, to avoid any further changes of the matrix.

Chemicals and Reagents. All reagents used were analytical reagent grade or higher. Ultrapure water (18 MΩ-cm resistivity) was freshly prepared with a MilliQ water purification system (Millipore, Saint-Quentin-en-Yvelines, France).

Fremy's salt solution: A solution of 2 mmol/L of Fremy's salt (potassium nitrosodisulfonate, Sigma Aldrich, St. Louis, MO) was prepared in phosphate buffer, pH = 7.4.

Trolox solution: A solution of 1 mmol (2.50 mg/10 mL) of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma Aldrich) was prepared in methanol and diluted for the measurements with water in the ratio 1:1.

Stock standards solutions for the LC/MS/MS measurements were prepared from solid native acrylamide (purity >99%, Sigma Aldrich) and solid isotopic labeled acrylamide (D_3-acrylamide, Cambridge Isotopes Laboratories, Andover, MA) by accurately weighing and dissolving in water. Working standards consisted of dilutions of the stock standard in the mobile phase of the HPLC method.

Acrylamide Determination by Liquid Chromatography−Tandem Mass Spectrometry (HPLC-MS/MS). The AA of 3 g of sample was extracted with 30 mL of water in an ultrasonic bath at 35 °C for 30 min. A 10 mL aliquot of the centrifuged extract was purified by Carrez precipitation. The final cleanup consisted of solid-phase extractions of 3 mL of sample first on OASIS HLB cartridges (6 mL, 500 mg, Waters, Milford, MA) and afterward on Isolute Multimode cartridges (3 mL, 500 mg, International Sorbent Technology, Hengoed, Mid Glamorgan, UK). An aliquot of 100 μL was injected on a Shodex RSpack 624L HPLC column (Showa Denko Europe, Munich, Germany). Chromatography was performed isocratically with a mixture of 40% methanol in 0.05% aqueous formic acid.

The quantification of AA was accomplished by HPLC-MS/MS on a Micromass Ultima PT mass spectrometer (Waters), with electrospray ionization in positive ion mode. AA was identified by multiple reaction monitoring (MRM) set to record the transitions m/z 72 > 72, 72 > 55, and 72 > 44 for AA and m/z 75 > 58 and 75 > 44 for D_3-AA.

Antioxidant Activity Determination by Electron Paramagnetic Resonance Spectroscopy (EPR). For the determination of the antioxidant activity of the samples, 2 g of the homogenized biscuits were extracted with 20 mL of warm water (∼40−50 °C). The slurry was mixed by means of a Vortex mixer and then shaken in a warm water bath for 15 min. Each sample was then cooled (4 °C) and centrifuged in order to obtain good phase separation. Finally, an aliquot of the aqueous extract was filtered through a 0.45 μm nylon filter. If necessary, samples were diluted with pure water in order to avoid overload of the detector. For calibration purposes, the Trolox solution was diluted in ratios of 1:2; 1:4, and 1:8 with methanol:water ratios of 1:1 to give final concentrations of 0.063−0.250 mmol L^{-1}. A 100 μL aliquot of sample solution or Trolox solution was mixed with 100 μL of the Fremy's salt solution of which 50 μL (volume of a standard capillary) was used for the electron paramagnetic resonance spectroscopic (EPR) measurements. They were performed on a benchtop EPR spectrometer (Mini Scope 200-Magnettech, Berlin-Adlershof, Germany) of high sensitivity for spin resonance spectroscopy.

The parameter settings were as follows: magnetic field 3360 G; sweep time 50 s (period of time in seconds needed for 1 scan); modulation amplitude 460 mG; microwave power 13 dB (corresponds to ∼5 mW); and a gain 2 (for amplification of the recorded signal). The EPR spectrum of the Fremy's salt radical was obtained after 20 min, by which time the reaction was complete. The velocity by which the signal decreases determines the antioxidant potential and the decrease in signal intensity provides a measure of the antioxidant capacity.

Color Measurements. Color measurements were carried out using a Chroma Meter CR-410 colorimeter (Konica Minolta, Mahwah, NJ) which recorded colors in the $L^*a^*b^*$ color space, devised in 1976 by the Commission Internationale de l'Eclairage (CIE), as a means of expressing color numerically (*27*). In this color space, L^* indicates luminance and a^* and b^* are the chromaticity coordinates. The value of a^* indicates the position between green and red (the latter at the positive end of the scale) and b^* the position between yellow and blue (the latter at the positive end of the scale). After calibration of the colorimeter using the instrument's reference white plate, color profiles of the samples were obtained by duplicate measurements of the cookie powders directly in the 50 mm diameter cell.

Nitrogen Determination. The nitrogen contents of the samples were determined by the Dumas method (*28*). About 400 mg of sample was burned in the combustion chamber of a VarioMax CN element analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The combustion parameters were as follows: combustion temperature 900 °C; CO_2 columns 250 °C; oxygen flows 140 or 70 mL min^{-1}; helium flow 690 mL min^{-1}; oxygen dosage time 50 s. To make the nitrogen contents more illustrative, protein contents were calculated by applying the nitrogen/protein conversion factor for wheat flour, irrespective of the contribution of egg proteins to the total protein content (*29*).

The values of the AA concentration and of the AOA were expressed on the basis of dry matter of the cookies, which was determined for each sample from the difference of weight after drying overnight at 103 °C. For a better comparability of the data, both parameters are further given in ng or nmol Trolox equivalents per g dry matter.

Statistical Evaluation of Data. All data were evaluated by applying different software packages such as Statgraphics Centurion beta version (StatPoint, Inc., Herndon, VA) and SPSS (SPSS Inc., Chicago, IL). All regressions were performed by applying the least-squares fit routine. The figures shown here were produced with SigmaPlot 2001 for Windows version 7.0 (SPSS Inc.).

RESULTS AND DISCUSSION

Influence of the Baking Time. The baking time showed a strong influence on both the AA formation and the AOA of the cookies. A direct correlation between baking time and AA content in bread was already highlighted (*30*). **Figure 1** depicts an example of the relationships between the AA content, the AOA, and the baking time of cookies which contained 37% w/w of sucrose. However, the results for the other self-prepared cookies were comparable. As can be seen, the increase of the AA content of the cookies, with the baking time, follows an exponential function, resulting in an 8-fold amount after 30 min compared to 15 min baking time. A similar relationship was found for the AOA of the cookies, which is expressed as Trolox equivalents per gram of sample (given on the left scale of **Figure 1**). The two graphs clearly indicate that the AA formation and the generation of antioxidants are linked together and even follow the same trend. The correlation coefficients for both regression curves were above 0.95. As for the AA content, the AOA increased as well by a factor of more than 4 in the aforementioned time interval. This means that just by changing the processing conditions to that of shorter baking duration will cause a decrease of the AA content, but at the cost of the AOA of the products. To check to what extent the AOA is caused by the AA content of the cookies, aqueous solutions of AA were included in the AOA determinations. None of the solutions that would represent an AA concentration of 500 ng/g cookie sample showed any antioxidant activity. The results for all samples are given in **Table 1**.

The baking temperature was kept constant in all experiments at 180 °C, thus providing the possibility to indicate differences of thermal load of the cookies by the baking time. Nevertheless, the evaluation of the results according to thermal load was not conclusive, if cookies produced with different pastries were compared. Within the series of cookies that were, e.g., produced according to recipe 2, only the absolute amount of sugar added to the pastry was different. Thus, concerning the AA content of the cookies, a direct correlation with the baking time could be expected. But as can be seen in **Table 1**, the AA contents as well as the AOA did not show any trend. This changed when considering the moisture content of the cookies in the evaluation of the data. The interpretation of the data, based on the moisture content of the samples, gave a much more conclusive picture than that based on baking time. It seemed that, despite constant settings, the baking conditions showed considerable variability, which then led to the variability in AA content and AOA.

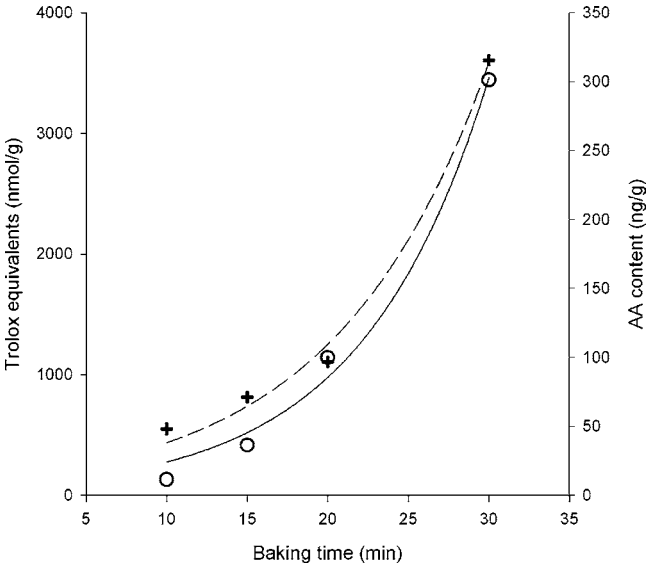

Figure 1. Influence of the baking time on the concentration of AA and on the AOA in recipe 2 cookies containing 37% (w/w) sucrose: (○) AA values; (+) AOA values; solid line, regression curve for AOA; broken line, regression curve for AA content.

The effect of the moisture content is particularly clear for the samples obtained with recipe 1. In this recipe, butter was replaced by cream and as a consequence the moisture content is approximately double as much as the moisture content in the cookies made with recipes 2 and 3 (having sucrose contents of 23 and 16% w/w, respectively). Those two resemble best recipe 1 in terms of sugar concentration. The highest AA concentration for recipe 1, obtained after baking for 20 min, was 63 ng g^{-1}, while for recipes 2 and 3 the concentrations of AA after baking for the same period of time were 37 and 406 ng g^{-1}, respectively. Regarding the moisture content, the samples obtained with recipes 1 and 2 were similar, while the samples produced with recipe 3 had approximately three-fourths less moisture. Such inverse correlation of AA formation and moisture content, as indicated in **Figure 2**, was also reported by Elmore et al. (*31*). They established a linear trend for the AA formation in the moisture content range between 0 and 4% w/w. In our investigations, which included a broader range of moisture contents, an exponential relationship was determined, which was remarkably independent of the sucrose content of the samples. This relationship is depicted in **Figure 2** for all samples containing sucrose. The fitted exponential function was tested by the measurement results for samples that were bought in local supermarkets. The moisture values of six of them agreed well with the established model (indicated in **Figure 2** by black dots). The commercial sample 7 (see **Table 1**) showed much higher AA contents, which were attributed to a different composition of the pastry; i.e., it contained fructose instead of sucrose. The correlation coefficient of the curve, fitted to the data of all cookies, is 0.75, which considers the number of samples and the high variability of the recipes and processing conditions better than expected.

On replacement of the AA content in **Figure 2** by the AOA, a similar graph is obtained, if just sucrose-containing samples are considered (not shown). It shows low AOA values at higher moisture contents and an exponential increase at low moisture contents. The correlation coefficient of the fitted model is 0.67. For the cookies containing fructose, the AOA was much more pronounced at low moisture contents, which resulted in a regression curve with a steeper slope. The correlation coefficient

Table 1. Protein, Moisture, and AA Content as Well as AOA Expressed in Trolox Equivalents of Samples Depending on Recipe and Baking Time

	baking time (min)	protein content (% w/w)	moisture content (% w/ w)	AA content (ng/g)	Trolox equivalents (nmol/g)		baking time (min)	protein content (% w/w)	moisture content (% w/ w)	AA content (ng/g)	Trolox equivalents (nmol/g)
	5	6.6	25.9	5	407		5	7.8	20.4	5	380
recipe 1;	10	7.3	18.4	8	349	recipe 3;	10	8.0	17.5	8	412
22% sucrose	15	7.5	14.2	15	872	0% sucrose	15	8.7	10.6	65	814
	20	8.4	6.6	63	2384		20	8.9	7.7	82	1570
	10	10.6	22.2	4	366		5	7.7	13.7	5	583
recipe 2;	15	12.0	13.1	51	605	recipe 3;	10	8.0	10.5	10	616
0% sucrose	20	12.5	10.3	63	698	9% sucrose	15	8.1	7.7	29	638
	30	13.5	3.9	330	2151		20	8.6	4.0	127	1744
	10	9.3	19.8	2	443		5	6.5	12.5	6	465
recipe 2;	15	9.8	13.6	6	616	recipe 3;	10	7.4	5.2	33	872
13% sucrose	20	10.8	7.7	65	1163	16% sucrose	15	7.2	4.1	47	930
	30	11.1	4.2	150	1686		20	7.9	1.6	406	3081
	10	8.6	13.0	4	581		5	5.5	13.4	3	465
recipe 2;	15	8.9	9.0	14	640	recipe 3;	10	5.9	7.6	10	872
23% sucrose	20	9.0	7.5	37	756	28% sucrose	15	6.2	5.2	28	930
	30	9.7	2.6	282	3314		20	6.3	3.5	82	1860
	10	7.5	10.1	12	603		5	7.0	17.1	11	640
recipe 2;	15	8.0	6.6	18	698	recipe 4;	10	7.8	9.3	211	1453
31% sucrose	20	8.0	4.3	73	1279	9% fructose	15	8.1	7.4	353	3081
	30	8.2	2.3	233	2093		20	8.2	4.0	487	16279
	10	6.9	7.3	11	550		5	6.7	14.6	17	465
recipe 2;	15	7.0	5.9	36	814	recipe 4;	10	7.0	10.3	181	2035
37% sucrose	20	7.4	3.3	100	1105	16% fructose	15	7.2	7.7	328	4244
	30	7.6	1.6	301	3605		20	7.6	6.1	466	25872
							5	5.4	14.6	12	465
						recipe 4;	10	5.8	8.5	165	4884
						28% fructose	15	5.8	6.7	291	5756
							20	6.4	2.8	428	28488

	protein content (% w/w)	moisture content (% w/ w)	AA content (ng/g)	Trolox equivalents (nmol/g)
commercial sample 1	7.5	2.7	313	2035
commercial sample 2	7.4	2.2	176	1512
commercial sample 3	9.5	4.7	81	1628
commercial sample 4	6.8	3.1	96	2151
commercial sample 5	5.5	4.6	65	814
commercial sample 6	6.8	3.8	209	1395
commercial sample 7	12.2	5.3	4108	8430

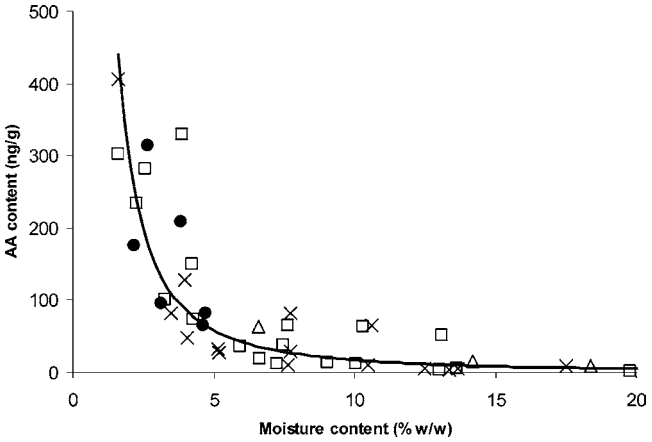

Figure 2. Correlation between moisture and AA content of the samples containing sucrose: (△) samples produced with recipe 1; (□) samples produced with recipe 2; (×) samples produced with recipe 3; (●) commercial samples.

of that curve was 0.85. The probability of a statistical significance of the correlation between moisture content and AA content respectively AOA was greater than 99%, which was indicated by *P*-values below 0.01.

According to Taeymans et al., a model for the reduction of AA in cereal-based products that relies solely on the modification of process parameters but simultaneously conserves other product properties has not been discovered yet (*32*). Unfortunately, the investigated product properties were not mentioned in detail. However, our findings support this statement with regard to the formation of antioxidants.

Influence of the Type of Sugar. Depending on the type of sugar, a different relationship between the AA content of the samples and the baking time was found. As illustrated in **Figure 3**, a linear increase was observed for the recipe in which fructose was applied, indicating first-order kinetics, while when sucrose was used, an exponential response was obtained. An explanation for this finding could be that fructose, which is a reducing sugar, reacts immediately with asparagine at a constant reaction rate. This is supported by comparable slopes of the regression curves of all three recipes containing fructose (not shown). For sucrose a certain heating and reaction time is needed to break the bond between glucose and fructose, releasing the two reducing sugars which would then participate in the Maillard reaction. Such a difference was not observed for the increase of the AOA. Both cookies containing sucrose and fructose showed an exponential increase of the AOA at increasing cooking time. However, the AOA formation was much higher for fructose-containing

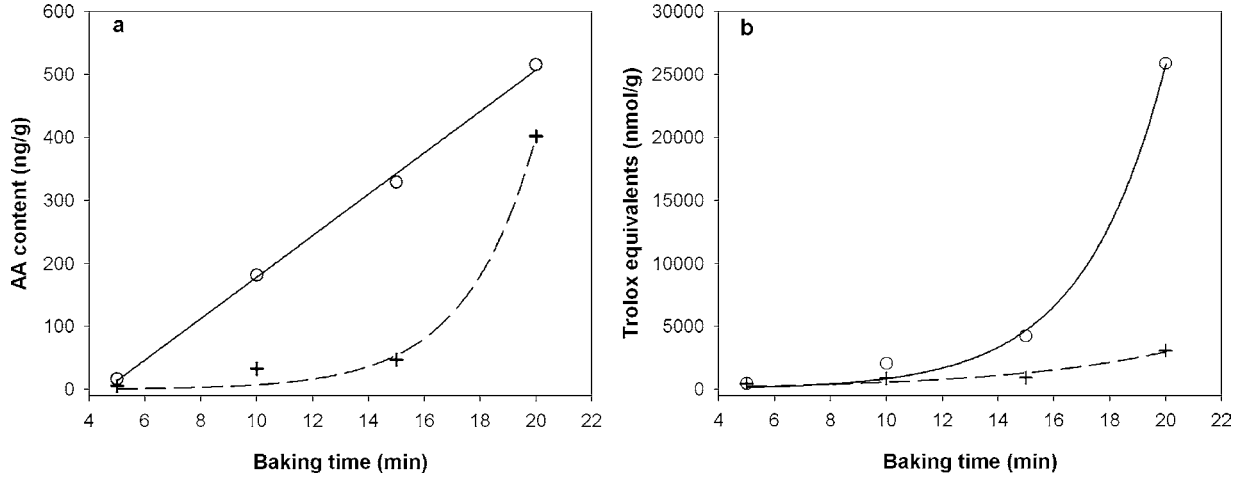

Figure 3. Effect of the type of sugar (fructose or sucrose) on (**a**) the concentration of AA and (**b**) the AOA. Sugar content: 16% (w/w): (○) samples containing fructose; solid line, regression curve for fructose cookies; (+) samples containing sucrose; broken line, regression curve for sucrose cookies.

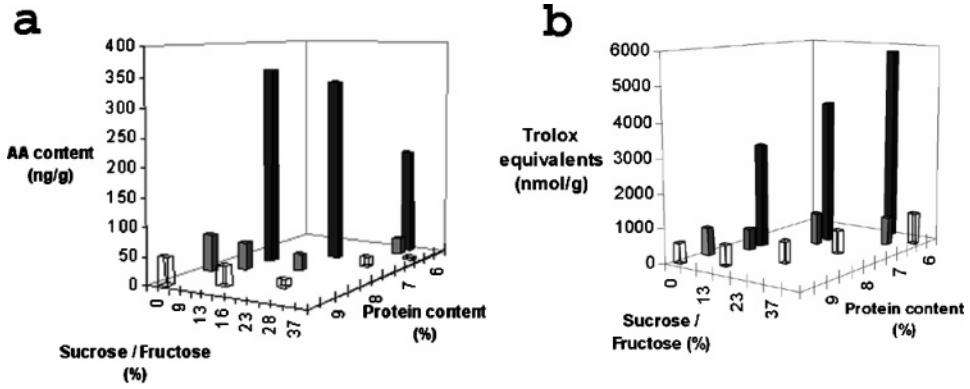

Figure 4. Simultaneous evaluation of the influence of the relative sugar and protein content on (**a**) the concentration of acrylamide and (**b**) the antioxidant activity. 15 min baking time for all samples: black columns, samples prepared with recipe 4; gray columns, samples prepared with recipe 3; white columns, samples prepared with recipe 2.

cookies compared to sucrose-containing cookies. This can be attributed to the presence of a reactive carbonyl function in fructose, which can react with a wide variety of compounds to form complex reaction products, which could contribute to the evolution of AOA (*19*, *20*).

The type of sugar used did affect not only the kinetics of the AA formation but also the absolute value of AA concentration. For otherwise similar compositions (recipe 3 and 4) the concentration of AA was always found to be higher when the pastries contained fructose (recipe 4) instead of sucrose (recipe 3). Only in the case of the 5 min baking times, similar but low AA concentrations were obtained (see **Table 1**), which could be explained by the still quite high water content that impairs the Maillard reaction.

The same applies to the AOA, although the effect in this case is much more pronounced. Equal values were obtained when baking for 5 min, but when the 20 min baking time data were examined, a 20-fold increase of the AOA was measured in fructose-containing cookies compared to those containing sucrose.

Influence of the Amount of Sugar. The influence of the amount of sugar added to the pastry on the AA content and the AOA of the cookies is different. The concentration of AA in the samples to which sugar was not added is among the highest, and it is even the highest after 30 min of baking. The moisture content for these samples is additionally, on average, higher than those for the other samples, which is in contradiction to the aforementioned relationship between moisture and AA formation. The reason for higher AA levels could be the higher relative concentration of proteins and especially of the amino acid asparagine in the pastries, when no sugar is added. It is known that the asparagine content is the limiting factor for the genesis of AA in bakery products. Fortification of the pastry with asparagine resulted in higher amounts of AA in the final products. Increasing the relative content of sugar in the pastry, as was done here, is equal to a dilution of the flour and thus in the concentration of proteins and asparagine (**Table 1**).

Asparagine is not a prerequisite for the formation of antioxidants. Other amino acids react as well with reducing sugars to form antioxidant MRPs (*33*). The greater complexity in terms of types of reactions and nature and amount of compounds formed makes it more difficult to establish a general rule to explain the results obtained. **Figure 4** shows the AA concentration and the AOA as a function of the relative amount of sugars and of proteins (calculated from total nitrogen) for recipes 2, 3, and 4, when baking for 15 min. Confirming our hypothesis, the concentration of AA decreases when the relative amount of proteins decreases (**Figure 4a**). This is very pronounced for fructose-containing samples (black bars). The tendency is the opposite for the AOA, showing that the limiting factor for the formation of antioxidants in the Maillard reaction is the concentration of sugars (**Figure 4b**).

Recipes 2 and 3 differed in the amount of butter and the use of commercial baking powder. However, these two ingredients caused a shift of the relative contents of sugar and flour. Therefore, a comparison of similar samples, e.g., containing 13%

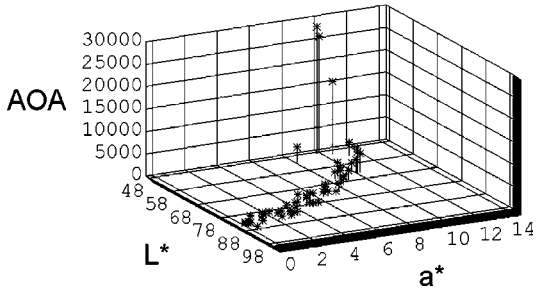

Figure 5. Correlation between AOA and the color of the samples: *L**, luminance; *a**, green-red value; vertical lines indicate the corresponding *L**/*a** values.

or 16% of sucrose, was possible. For identical baking times, the concentration of AA in the cookies prepared with recipe 3 was higher than in those prepared following recipe 2, although the protein content of the former was slightly lower (8.7−5.2% w/w) than that of the latter (12.0−7.0% w/w), indicating again the complexity of the Maillard reaction. An explanation could be that the moisture content of the samples made with recipe 3 was lower (10.6−5.2% w/w) than that of cookies prepared with recipe 2 (13.1−5.9% w/w). Also, the fat content in recipe 3 is substantially higher than that in recipe 2, which could promote alternative reactions to form AA. One such reaction mechanism for the formation of AA, from triglycerides, with acrolein as an intermediary, has been described for lipid-rich foods, although the studies have been performed on vegetable oils and not on butter (*34*, *35*).

The use of baking powder has shown to have an effect on the AA generation too, but only when it contains ammonium bicarbonate (*36*). The baking powder used in this study contained starch, Na_2HPO_3, and $NaHCO_3$; thus, its influence in the formation of AA cannot be stated (*37*).

Correlation between Antioxidant Activity, Acrylamide Concentration, and Color of the Samples. A correlation was established between the AOA and the color of the final homogenates. **Figure 5** contains AOA and color data of 40 different cookie samples, data of 12 samples containing fructose, and data of 6 commercial cookie samples containing sucrose. Although the CIE color space is composed of three parameters that are linked to each other, only two of them were clearly changed by the processing of the cookies. The green-red parameter *a** showed a direct correlation to increasing AOA for all samples. That means increasing levels of AOA were accompanied by a displacement of the color of the homogenate toward brown. Above a certain level, the brown tone did not change any more, while the AOA still increased very much. Simultaneously, the AOA was indirectly correlated with *L**-values (luminance), so the higher the AOA, the darker the samples. The AA content of the samples showed a similar trend. Just the variability of the data at very dark brown tones was higher compared to the AOA values The respective correlation coefficients of the regression curve for the correlation of *L** and AOA was 0.82, indicating that the model as fitted explains 82% of the variability of the AOA data, while correlating *L** and AA contents resulted in a correlation coefficient of 0.62. It is not clear if this enhanced variability of the AA results was a consequence of the model itself or if already other reactions influenced the AA content since the samples with high AA values were already very dark brown. There are reports claiming an important contribution of caramelization reactions on the nonenzymatic browning (*39*). Consequently, this additional color component could lead to overestimations of the AA content, if all browning is exclusively attributed to the Maillard reaction. However, previous publications focused on the relationship between one CIE parameter and the AA content of the samples (*38*, *40*). Our studies revealed that the relationship between color and the AA content, respectively AOA, could be better expressed by a three-dimensional model.

However, the validity of the model is underlined by the good agreement of the data of model cookies with the data for commercial samples.

Determination of Acrylamide and Antioxidant Activity in Commercial Cookies. Cookies of seven different brands purchased at local supermarkets were analyzed. Invariably it was observed that the higher the AA concentration, the higher the AOA, confirming the results previously described. For a given set of protein and moisture contents, the AA and AOA values are in the same range as found for the model cookies (**Table 1**). Of particular interest is sample 7. It represents diet cookies, prepared with fructose and whole wheat flour. The AA content was by far the highest in the whole series of experiments, much higher compared to that of self-prepared fructose-containing model cookies. However, the AOA was in the same range as that found for model cookies. An explanation for the high AA values could be the high protein content of sample 7, which was about double that of the samples under comparison, supporting the findings that the AA formation is directly linked to the protein/asparagine content of the samples. Additionally, whole flour enhances the AA genesis in cookies compared to plain flour (*37*).

However, the results obtained for commercial samples confirmed the findings for model cookies. It turned out that there is a strong link between the composition of the pastries, baking parameters, and the genesis of AA as well as antioxidants. Suppressing the Maillard reaction in general leads not only to AA mitigation but also to losses of substances considered to be beneficial.

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