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# Role of Water upon the Formation of Acrylamide in a Potato Model System

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The moisture sorption isotherms of a commercial potato powder were investigated at 20 °C for water activities ranging from 0.11 to 0.97. The sorption isotherms were typical type-II sigmoidal curves, with a steep increase in moisture content for water activities above 0.9 and exhibiting hysteresis over the whole water activity range. On the basis of the isotherms, the influence of the initial water activity and moisture content on both Maillard browning and acrylamide formation was determined by heating oil containing potato powder mixtures in a closed stainless-steel tubular reactor. The Maillard browning, as determined spectrophotometrically, showed an optimum at intermediate water activities. The yields of acrylamide, expressed relatively to the molar amount of asparagine, remained constant below 0.8 $a_w$ and below moisture contents of about 20% (on a dry basis). For the more intense heat treatments, an increased acrylamide yield was however observed at higher moisture contents, with an optimum at water contents of about 100% (on a dry basis). However, this increase and optimum was not observed at less intense heat treatments. At moisture contents above 100%, a significant decrease in acrylamide yields was assessed, although the water activity increased only marginally in this area of the sorption isotherms. It was thus observed that the acrylamide content was rather dependent upon the moisture content than upon the water activity in the high-moisture potato powder model system.

**KEYWORDS: Sorption isotherms; hysteresis; water activity; acrylamide; Maillard browning; LC−MS/MS**

## INTRODUCTION

Fried potatoes are widely consumed, because they exhibit appealing sensory quality characteristics to consumers. However, these foodstuffs pose some chemical safety hazards. Because deep frying is conducted at elevated temperatures, this leads to the formation of acrylamide, which is classified as probably carcinogenic to humans (*1*). The main formation pathway of acrylamide is related to the Maillard reaction (*2*, *3*), starting from reducing sugars and free asparagine (*4*, *5*). Recently, also 3-aminopropionamide was proven to be an important acrylamide precursor (*6*).

Deep frying has been defined as the submersion of a food product in an edible oil or fat heated above the boiling point of water. Therefore, this operation may be considered a dehydration process, which comprises the evaporation of water from the product, in the form of vapor. Simultaneously, oil penetrates the product, and major transformations in the (porous) microstructure occur, which determine the final physical and sensorial food properties. In addition, chemical interactions between food components and the frying oil give rise to nonvolatile and volatile compounds (*7*, *8*). The nonenzymatic browning reaction (Maillard reaction) generates a vast range of odor and flavor molecules, as well as brown pigments (*9*).

Furthermore, it has been shown that water activity ($a_w$) is a key factor to consider in the Maillard reaction (*9*−*11*). Optimal rates of Maillard browning at intermediate $a_w$ are reported (*9*). At lower water activity levels, the molecular mobility or solubility is hindered. At higher levels, the reaction rates decrease because of a dilution effect of the reactants. Because water is produced during the Maillard reaction, the law of mass action plays an inhibiting role, as well at high $a_w$. The consistency of other components within the matrix, such as polymers and humectants, may also affect the mobility of the reactants, complicating the unravelling of the Maillard reaction and the factors influencing it (*9*, *11*, *12*).

The above-mentioned optimal rate of browning can best be explained by reference to the moisture sorption isotherms. Moisture sorption isotherms represent the relationship between the equilibrium moisture content and water activity at constant temperatures and pressures (*13*). Because food materials have

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complex compositions and structures, sorption isotherms actually describe the integrated hygroscopic properties of the various constituents. They express the sorption mechanism and the interaction of food biopolymers with water (*13*).

Because acrylamide formation is linked to the Maillard reaction (*2*, *3*), water activity and moisture content may also have an impact on the generation of acrylamide. Sadd and Hamlet (*14*) developed a mathematical dough model, which showed that acrylamide formation increased upon decreasing the moisture content. However, the Maillard reaction showed an optimal level of browning at intermediate moisture levels, indicating that the Maillard reaction and acrylamide formation do not always concur. The impact of the water activity was however not investigated in this study. On the other hand, Robert et al. (*10*) proved that $a_w$ is not a critical parameter for acrylamide formation in low-moisture model systems ($0.07 < a_w < 0.22$) based on asparagine and glucose. However, the acrylamide amounts were correlated with physical changes occurring during the reaction, such as the melting of the sugars, and the physical state of the reaction system (crystalline versus amorphous).

On the other hand, model studies of Schieberle et al. (*15*) revealed a dramatic drop of acrylamide yield when lowering the water content of the heated mixture from 10 to 0%. The yields further increased when experiments were performed at a water content of 25% but dropped again when tests were performed in an aqueous buffer.

Recently, a kinetic study was published (*16*) in which the influence of the moisture content on the color and acrylamide formation in a closed potato model system was investigated at three heating temperatures (119, 143, and 167 °C). At low- and high-moisture contents, lower Maillard browning and acrylamide formation rates were determined. Increasing activation energies for both parameters at low-moisture contents (<25 g/100 g of dry matter) were demonstrated. Also here, the impact of $a_w$ was not considered.

In this study, the relationship between the water activity and moisture content of a commercial potato powder was investigated through the construction of sorption isotherms. In such a way, a better insight into the integrated hygroscopic properties of the foodstuff could be acquired. On the basis of this knowledge, the subsequent step was to determine whether initial water activity or water content was the crucial factor on the formation of brown Maillard pigments and on acrylamide generation in a closed and heated potato powder model system.

## MATERIALS AND METHODS

**Materials.** Potato powder was purchased in retail (Unilever, Brussels, Belgium). The powder was sieved, and the fraction between 90 and 160 $\mu$m was retained. The initial dry matter content and water activity was 90.33 ± 0.24% and 0.37 ± 0.015, respectively. The fructose, glucose, sucrose, and asparagine contents were 0.0300, 0.0335, 1.0721, and 0.8889 g/100 g of powder, respectively (*17*, *18*).

**Sorption Experiment.** For the determination of the adsorption isotherm, the potato powder was initially dried for about 48 h in a vacuum oven at 30 °C until no appreciable weight loss was noted. Sulfuric acid (96%; Chem-lab, Lichtervelde, Belgium) was used to trap water vapor inside the oven. The powder was thereupon used for the development of the adsorption isotherm by the static gravimetric method. For the desorption isotherm, the potato powder was initially placed over pure distilled water until no appreciable weight gain was observed before use. Pure distilled water and nine saturated salt solutions of different relative humidity were selected to get different $a_w$ values in the potato powder, ranging from 0.110 to 0.972. The relative humidity/$a_w$ values of the salt solutions were taken from Labuza (*19*), McLaughlin and Magee (*20*), and Resnik and Chirife (*21*) and include LiCl, $CH_3COOK$, $MgCl_2$, $K_2CO_3$, NaBr, NaCl, KCl, $BaCl_2$, and $K_2SO_4$ (purity > 99%; Acros Organics, Geel, Belgium). About 10−15 g of potato powder was placed in a glass jar ($V$ = 2.5 L) containing a saturated salt solution or distilled water. An internal platform was used to raise the samples off the floor. Experiments were carried out in triplicate. A small amount of toluene was placed in each jar to prevent the growth of fungi (*19*, *22*). The jars were then placed in a constant temperature cabinet at 20 ± 1 °C.

**Table 1.** Isotherm Equations for Experimental Data Fitting

| model name | equation |
|---|---|
| Peleg (*25*) | $M = K_1 a_w^{n_1} + K_2 a_w^{n_2}$ |
| GAB (*26*) | $M = m_0 C K a_w / [(1 - K a_w)(1 - K a_w + C K a_w)]$ |
| Halsey (*24*) | $a_w = e^{(-k/M^n)}$ |

$K_1$, $K_2$ = equation parameters
$n_1$, $n_2$ = equation parameters
$a_w$ = water activity
$M$ = moisture content (g/100 g of dry matter)
$m_0$ = monolayer moisture content (g/100 g of dry matter)
$C$ = GAB model parameter
$K$ = GAB model parameter
$k$, $n$ = constants

The samples were left to equilibrate until the weight was constant over at least three consecutive days. The total weighing time was maintained at less than 30 s to reduce the sorption of atmospheric moisture. The dry matter content of the potato powder was determined by the oven-drying method (*23*), and the exact value of the corresponding $a_w$ was confirmed by the Novasina Thermoconstanter TH200 (Axair Ltd. Systems, Pfapfikkon, Switzerland).

**Modeling of Sorption Isotherms.** The mathematical sorption isotherm models, shown in **Table 1** (*24*−*26*), were fitted to the experimental data. These include one four-parameter Peleg equation, one three-parameter equation (Guggenheim−Anderson−de Boer, GAB), and one two-parameter equation (Halsey). These sorption models are among those most widely used to describe sorption isotherms for various food materials. The parameters of the sorption models were estimated using the nonlinear regression function of SPSS version 12.0 (SPSS, Inc., Chicago, IL). The goodness of fit of the models was evaluated by means of the mean relative percentage deviation modulus ($P$), defined as

$$P(\%) = \frac{100}{N} \sum_{i=1}^{N} \frac{|M_{ei} - M_{ci}|}{M_{ei}}$$

where $M_{ei}$ and $M_{ci}$ are the experimental and predicted moisture content values, respectively, and $N$ is the number of experimental data. A model is considered acceptable if it has a $P$ value of less than 10% (*27*).

**Preparation of Homogeneous Artificial Mixtures.** Immediately after concluding the water activity measurements, the 10 potato powders, having a different $a_w$, were mixed with sunflower oil in a mortar. The total mixing time was maintained at less than 1 min to reduce the sorption of atmospheric moisture. The final oil content of all mixtures was fixed at 21% (w/w). Besides the static gravimetric method, during which the potato powder adsorbed or desorbed water through the vapor phase, potato powder mixtures were also prepared by adding distilled water to the dry potato powder. Sunflower oil was also added to obtain a similar final oil content after mixing in a mortar.

**Heating Experiments.** A total of 1 g of the homogenized mixture was introduced as a cylinder into the middle of a cylindrical stainless-steel tubular reactor (internal diameter of 1 cm and length of 30 cm), as described earlier (*18*, *28*). The mixture was kept in place by two stainless-steel supporting bars (diameter of 1 cm), which were introduced at both sides of the stainless-steel tube. Then, the reactor was hermetically sealed and heated in a deep-fryer (Fritel 2505, Hasselt, Belgium), equipped with a thermocouple (Testo 925, Ternat, Belgium) and with a stirring mechanism to ensure a homogeneous temperature

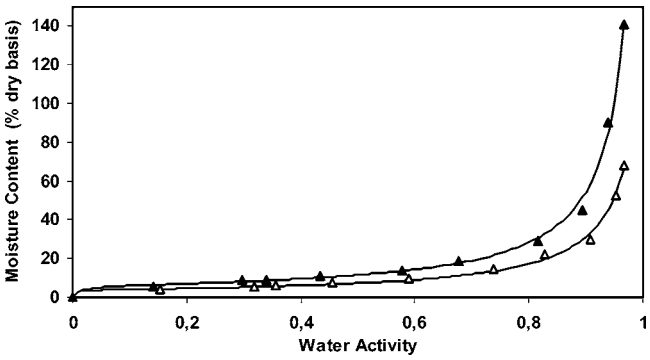


**Figure 1.** Adsorption (△) and desorption (▲) isotherm of potato powder at 20 °C. The smooth curves represent the GAB isotherm curves, fitted to the data.

in the oil bath. Heating experiments were performed for 3, 5, and 7 min at an oil bath temperature of 170 (±1) °C. After heating, a quick cooling was established, submerging the reactor in an ice bath for 2 min. Finally, the 1 g mixture was analyzed for its acrylamide content.

**Acrylamide Analysis.** The LC−MS/MS analysis method is similar to the one described earlier (*28*). Briefly, homogenized samples, spiked with 40 $\mu$L of 10 ng/$\mu$L [2,3,3-$D_3$]acrylamide (Polymer Source, Inc., Dorval, Canada), were defatted with 10 mL of *n*-hexane followed by 10 min of shaking. Acrylamide was extracted using 10 mL of deionized water, followed by a 20 min shaking period, centrifugation, and ultrafiltration through a 0.45 $\mu$m membrane filter. A clear-colored acrylamide extract was obtained accordingly. Half of the aqueous extract was kept for color analysis, as discussed further. On 2 mL of the remaining extract, further sample cleanup was performed on two solid-phase extraction columns (Oasis HLB, 6 mL, 200 mg; Waters, Milford, MA, and Bond Elut-Accucat, 200 mg mixed-mode packing: $C_8$, SAX, and SCX, Varian, Harbor City, CA). The extract was thereupon analyzed using LC−MS/MS with positive electrospray ionization. The method was validated in-house, as described in a previous paper (*28*). An external calibration curve was established in the concentration range between 0 and 10 000 $\mu$g kg$^{-1}$. Data interpretation was performed by use of the Quanlynx integration software (Micromass, Manchester, U.K.). Calibration curves were linear ($r^2 > 0.999$). The limit of detection (LOD), defined as the mean value of the matrix blank readings plus 3 standard deviations (expressed in the analyte concentration), was 12.5 $\mu$g kg$^{-1}$. The limit of quantification (LOQ), being the mean value of the matrix blank readings plus 6 standard deviations, was 25 $\mu$g kg$^{-1}$. The repeatability of the analysis method, expressed as the variation coefficient, was 10%. The 95% confidence intervals, presented between brackets in **Figures 3−5**, are based on repeatability experiments reported previously (*28*), yielding a variation coefficient of 12%. The molar acrylamide yields in **Figures 3−5** are expressed relatively to the molar amount of asparagine, present in each mixture.

**Color Analysis.** The color of the clear aqueous acrylamide extracts, obtained after 20 min of shaking, centrifugation, and ultrafiltration, was measured at 420, 450, 470, and 490 nm using a Varian Cary 50 Bio spectrophotometer (Mulgrave, Victoria, Australia) (*11*, *12*, *29*). The samples were diluted to obtain absorbance values mainly between 0.1 and 1.0. Absorbances versus different sample masses (0.50−1.25 g) of the same heated sample gave a linear relationship ($r^2 > 0.99$). The results in **Figure 2** are expressed in relative absorbance, being the absorbance of the clear extract divided by the amount of dry potato powder present in each mixture.

## RESULTS AND DISCUSSION

**Sorption Isotherms.** The adsorption and desorption isotherms of the potato powder at 20 °C are shown in **Figure 1**. The isotherms have a sigmoidal shape, depicting an increase in the equilibrium moisture content with $a_w$. This is typical for type-II isotherms and has been reported for starchy products such as potatoes (*20*, *22*, *30*), cookies, and corn snacks (*31*). Both adsorption and desorption isotherms can roughly be divided into two regions. In the region with $a_w < 0.9$, the moisture content only changes limitedly and reaches about 30% (on a dry basis). However, a steep increase in the moisture content, up to 140%, is assessed for $a_w > 0.9$, both for the adsorption and desorption isotherms (**Figure 1**), because of the increasing amount of free water in the mixture. Hysteresis can also be observed over the total water activity range investigated, because the equilibrium moisture content for desorption is higher than that for adsorption.

**Table 2.** Estimated Parameters and *P* (%) Values of the Sorption Equations Fitted to the Adsorption and Desorption Isotherm Data of the Potato Powder

| model | constants | adsorption | desorption |
|---|---|---|---|
| Peleg | $K_1$ | 25.703 | 26.761 |
| | $K_2$ | 113.601 | 226.583 |
| | $n_1$ | 1.499 | 0.980 |
| | $n_2$ | 27.638 | 19.971 |
| | ***P* (%)** | **7.88**[a] | **7.89** |
| GAB | $m_0$ | 3.747 | 5.867 |
| | $C$ | 473.169 | 91.027 |
| | $K$ | 0.977 | 0.992 |
| | ***P* (%)** | **8.53** | **7.96** |
| Halsey | $k$ | 13.218 | 12.062 |
| | $n$ | 1.411 | 1.186 |
| | ***P* (%)** | **3.32** | **9.19**[a] |

[a] For $a_w > 0.3$.

**Fitting Mathematical Sorption Models to Isotherm Data.** **Table 2** shows the coefficients of the three sorption isotherm equations fitted to the experimental adsorption and desorption data, respectively, and *P* (%), the mean relative percentage deviation modulus. Other sorption isotherm equations were also considered; however, only the equations best fitting to the experimental data are mentioned here. For the adsorption data, the Halsey, Peleg, and GAB model give a *P* value below 10% and can therefore be considered to be adequate for describing experimental adsorption data for the potato powder (*27*). For the desorption data, the Peleg equation shows the lowest *P* value, closely followed by the GAB and Halsey equation. Because the *P* value for the Halsey equation for the desorption data was initially slightly above 10% (10.21%), $a_w$ values below 0.3 were omitted upon fitting the data. The omission of extreme water activity points was already done previously (*32*) to obtain a better mathematical fit. Doing so, a *P* value below 10% was obtained. This was also done for the Peleg equation of the adsorption data, which had initially a *P* value of 10.66%. It should be realized that no single mathematical model can be considered accurate over the entire $a_w$ range, because water is associated with the food matrix by different mechanisms in different $a_w$ regions (*33*). Moreover, the goodness of fit of a sorption model to experimental data does not describe the nature of the sorption process. It only reflects on the mathematical quality of a model (*33*).

The GAB (plotted in **Figure 1** as a smooth line for both adsorption and desorption data) (*20*, *22*, *32*) as well as the empirical Peleg equation (*32*) have already been previously shown to be good models for predicting potato and potato starch isotherms. However, the findings for the Halsey model are somewhat contradictory. Al Muhtaseb et al. (*32*) and Wang and Brennan (*34*) have found the Halsey model to be inadequate for representing the sorption isotherms for starch powders and potatoes, respectively. This is in contrast to McMinn and Magee (*22*) and Kaymak-Ertekin and Gedik (*13*).

The estimated monolayer moisture content ($m_0$) from the adsorption isotherm using the GAB equation was 3.747 g/100 g of dry matter. A slightly higher monolayer moisture content

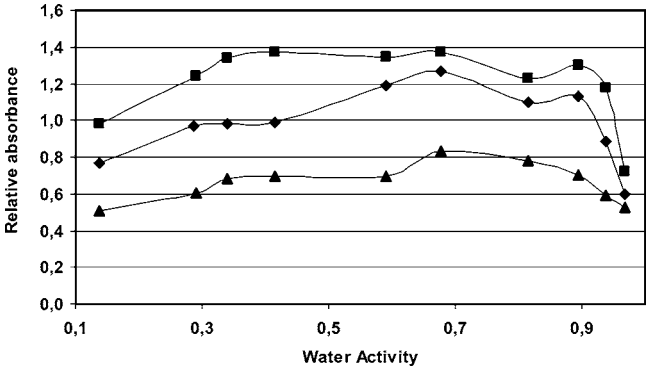


**Figure 2.** Relative absorbance values (420 nm), expressed relatively to the amount of dry potato powder, of the aqueous extracts of the potato powder mixtures, heated at 3 (▲), 5 (◆), and 7 (■) min.

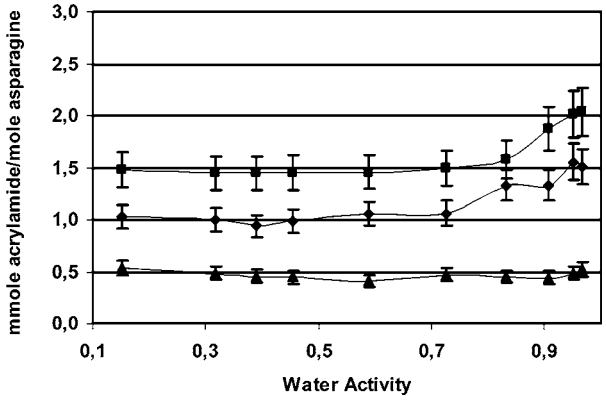


**Figure 3.** Effect of the initial water activity on the molar yield of acrylamide in homogeneous potato powder mixtures, obtained after a water adsorption experiment. The mixtures were heated for 3 (▲), 5 (◆), and 7 (■) min in a closed tubular reactor.

of 5.867 g/100 g of dry matter was determined from the desorption isotherms. The estimated values are comparable to the values reported by Al Muhtaseb et al. (*32*), being 3.1 and 5.6 g/100 g of dry matter, respectively, for adsorption and desorption isotherms of potato starch powder at 30 °C. The values are somewhat lower compared to those reported by McMinn and Magee (*22*) and McLaughlin and Magee (*20*) for potatoes. However, $m_0$ values between 3.2 and 16 g/100 g of dry matter have been reported for starchy foods (*35*).

**Influence of the Initial Water Activity on Maillard Color Formation.** To investigate the influence of initial $a_w$ on the Maillard reaction, the potato powders with different $a_w$, obtained from the adsorption and desorption experiments, were heated in a hermetically sealable tubular reactor. This was done to obtain constant food moisture content during heating, by eliminating water evaporation. It should however be realized that the measured initial $a_w$ is not constant during the heating process, because $a_w$ changes with temperature. The increasing vapor pressure in the sealed reactor upon heating additionally influences $a_w$. It can however be assumed that the course of this change upon heating is similar for each mixture. Consequently, a difference in $a_w$ remains between mixtures with distinct initial water activity levels at a specific point of the heating process. Sunflower oil was mixed with the different potato powders to obtain an oil content of 21% (w/w). In such a way, a more realistic mixture composition compared to, e.g., French fries was obtained without influencing $a_w$. To check possible sorption of atmospheric moisture upon mixing potato powder and oil, $a_w$ values of three potato powders (0.14, 0.44, and 0.94) before and after oil addition were measured. These differences were <0.005. The heating experiments were carried out at 170 °C for 3, 5, and 7 min.

The basis for the assessment of the rate of the Maillard reaction has been to monitor color formation as assessed in an aqueous extract, containing the water-soluble melanoidins (brown, nitrogenous polymers and copolymers), which are the final products of the Maillard reaction. Previously, the absorbance of heated reaction mixtures was determined spectrophotometrically at different wavelengths (420, 450, 470, and 490 nm) to assess the degree of the Maillard reaction (*12, 29*).

**Figure 2** shows the relative absorbance of the different potato powder mixtures, obtained from the construction of the desorption isotherm, for the three heating times. Only the 420 nm measurements are plotted, because the measurements at other wavelengths give a similar trend and lower abundance. To correct for the different moisture content between the heated mixtures, the results are expressed on the amount of dry potato powder. The relative absorbance values in **Figure 2** increase with increasing heating times and thus progressive Maillard browning. A maximum is observed around 0.7 $a_w$ for 3 and 5 min heating at 170 °C. For 7 min heating, the highest relative absorbance value is already reached at 0.4 $a_w$ and remains at this level until 0.7 $a_w$. In addition, a decrease in the color formation occurs at lower ($a_w$ < 0.3) and higher ($a_w$ > 0.9) initial $a_w$ levels. This shows that a difference in the initial water activity of the potato powders has an impact on the Maillard reaction in the closed tubular reactor upon heating. These results confirm previous studies in which an optimum of Maillard browning was observed at intermediate water activities (*9, 11, 12*). In addition, Sadd and Hamlet (*14*) also found optimum browning at intermediate moisture contents in a closed dough model system. However, in a closed potato model system, Amrein et al. (*16*) found optimum browning rates at moisture contents of about 68.4 g/100 g of dry matter. On the basis of the developed sorption isotherms, this roughly corresponds to 0.9 $a_w$. In this particular study, Maillard browning was however measured at the product surface as relative brightness ($L/L_0$), which may explain the different experimental outcome.

**Influence of the Initial Water Activity and Water Content on the Amounts of Acrylamide.** The results presented above show that the Maillard reaction and color formation in the model system have an optimum at intermediate $a_w$. Because acrylamide is linked to the Maillard reaction (*2, 3*), the impact of initial $a_w$ on the final acrylamide concentration was investigated accordingly. For this, the mixtures with different initial $a_w$, which were heated inside the reactor as discussed above, were analyzed on their acrylamide content. The acrylamide concentrations of the heated potato powders originating from both the adsorption and desorption isotherms (**Figure 1**) were determined. Because acrylamide is formed from the amide side chain of asparagine (*5*), the molar amounts of acrylamide in **Figures 3** and **4** are expressed relatively to the molar amounts of asparagine, present in each heated mixture. In such a way, the molar yield of acrylamide was calculated as a function of the initial water activity and water content. The results represent the average of three replicates.

This experimental setup did however not allow for a quantification of acrylamide degradation upon heating. However, **Figures 3** and **4** show that acrylamide contents rise upon increasing heating times. Because no decreasing trend for the longer heating times is assessed, this indicates that formation still exceeded elimination (*16*). In addition, the acrylamide levels

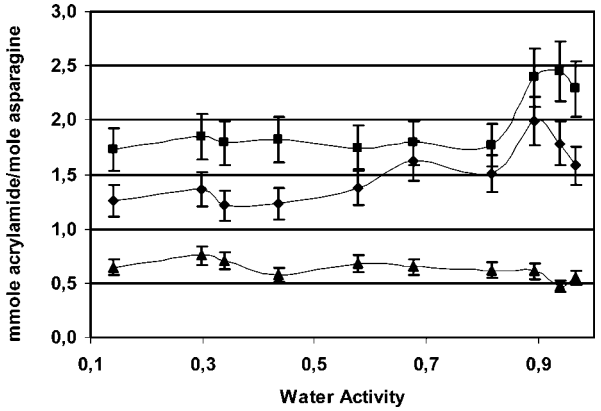


**Figure 4.** Effect of the initial water activity on the molar yield of acrylamide in homogeneous potato powder mixtures, obtained after a water desorption experiment. The mixtures were heated for 3 (▲), 5 (◆), and 7 (■) min in a closed tubular reactor.

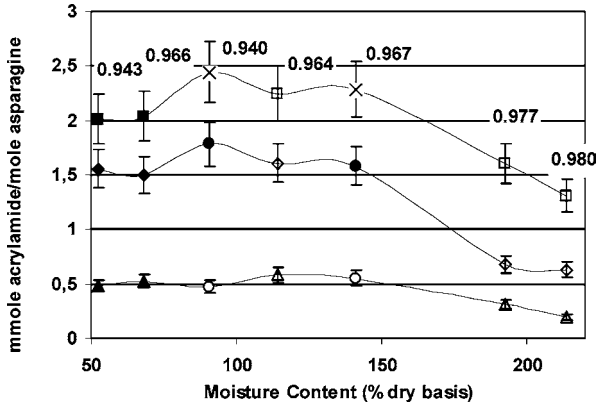


**Figure 5.** Effect of the high-moisture content (>50%) on the molar yield of acrylamide in homogeneous potato powder mixtures, containing different amounts of water. The mixtures were heated for 3 (△), 5 (◇), and 7 (□) min in a closed tubular reactor. Data from the adsorption experiment are also plotted for 3 (▲), 5 (◆), and 7 (■) min of heating. The desorption experimental data, with initial $a_w$ values of 0.940 and 0.967, are included as well, for 3 (○), 5 (●), and 7 (×) min in a closed tubular reactor. The labels show the water activity level corresponding to the moisture content of each mixture.

in **Figure 4** tend to be higher than those in **Figure 3**, although these differences are not significant. Furthermore, it can be observed that the final acrylamide yield in the heated potato model systems, originating from both the adsorption and desorption experiments, remains constant between 0.14 and 0.8 $a_w$. Robert et al. (*10*) also concluded that $a_w$ did not seem to be a critical parameter for acrylamide formation in asparagine/glucose model systems under low-moisture conditions ($0.07 < a_w < 0.22$). Even initial $a_w$ levels higher than 0.8 barely change the acrylamide yields in **Figure 3** for the mixtures heated for 3 min at 170 °C; in fact, a small decrease is observed at 0.94 $a_w$ in **Figure 4**. For the mixtures heated for 5 and 7 min, however, the acrylamide yields in **Figures 3** and **4** clearly increase with an increasing initial $a_w$ above 0.8. In potato mixtures obtained after the water desorption experiment (**Figure 4**), this increase is followed by a decrease in acrylamide yields when the initial $a_w$ of the mixtures further approaches 1.0. However, this reduction is not obvious in the heated mixtures, obtained after the water adsorption experiment (**Figure 3**). Consequently, it is clear that, depending upon the adsorption/desorption status of the potato powder, the acrylamide yields upon heating are different, despite the fact that the initial $a_w$ of the powder is the same. When evaluating these data against the sorption isotherms (**Figure 1**), it becomes clear that the powders experiencing water desorption have a much higher moisture content compared to the powders going through a water adsorption phenomenon, for similar $a_w$. This hysteresis effect is much more pronounced at $a_w > 0.9$. A slight increase in $a_w$ causes a steep increase in the moisture content in this region of the desorption isotherm, leading to a decrease in the final acrylamide yield upon heating (**Figure 4**). This acrylamide lowering effect at $a_w > 0.9$ is not observed for the heated mixtures originating from the adsorption isotherm (**Figure 3**), because moisture contents do not reach the same level for the investigated $a_w$ levels. Consequently, it is most likely that the moisture content, rather than $a_w$, plays a role in acrylamide formation in the current experimental setup.

To further study the impact of initial $a_w$ and water content on the acrylamide yield upon heating at a higher $a_w$ and water content range, the evolution in the acrylamide concentration was evaluated in heated potato powder mixtures with moisture contents above 100% (expressed on dry potato powder). For this, different amounts of distilled water were added to the dry potato powder. Three mixtures were prepared accordingly, with moisture contents of 114.1, 192.5, and 213.5% (expressed on dry potato powder) and water activities of 0.964, 0.977, and 0.980, respectively. These high-moisture content levels could not be achieved during the adsorption experiment, using the static gravimetric method (**Figure 1**).

Because this experiment can be considered as an adsorption process, the three points were evaluated against the adsorption isotherms (**Table 2**). Using the three measured $a_w$ values, these equations resulted in moisture content values of about 65, 83, and 90%, respectively, for the three potato powder mixtures. Consequently, it was observed that the measured moisture content values were situated above the predicted adsorption curves. In contrast to the static gravimetric method, however, the three mixtures were only left overnight to equilibrate between the addition of water and the $a_w$ measurement. The static gravimetric method indeed allows the potato powders 3 weeks to equilibrate above saturated salt solutions, before measuring $a_w$. In addition, the different manner of water administration to the potato powder could be another reason for this different sorption behavior. In the static gravimetric method, water adsorption occurs through the vapor phase, while for the second method, liquid water is added. It was stated as well (*33*) that not any model can correctly predict the sorption isotherm over the whole range of water activities, because water is associated with the food matrix by different mechanisms in different $a_w$ regions. Therefore, it could indeed be possible that the calculated adsorption equations are not valid for water activities above 0.96.

To the three potato powders obtained accordingly, 21% of sunflower oil was added, followed by homogenization. Similarly to the previous experiments, the mixtures were thereupon heated in the closed tubular reactor for 3, 5, and 7 min. **Figure 5** shows the molar acrylamide yields, together with the acrylamide yields originating from the two highest $a_w$ points of the adsorption and desorption isotherm experiments, respectively. Because $a_w$ levels only vary marginally in this region of the sorption isotherm (**Figure 1**), the data are now plotted against the moisture content, expressed in terms of percentage on the amount of dry potato powder. The water activity of each mixture is mentioned as labels (0.943−0.980). From this figure, it is clear that the acrylamide yields further drop as the moisture content further increases above 100%, confirming the decreasing trend in the acrylamide yield for high $a_w$ levels in **Figure 4**. This decreasing trend is now also visible for the high-moisture

content (>150%) mixtures heated for 3 min. From **Figure 5**, it is also clear that the formation of acrylamide is more likely dependent upon the moisture content rather than $a_w$.

In addition, the absorbance at 420 nm of the aqueous acrylamide extracts was measured, for the three heat treatments. A further reduction in absorbance was assessed, confirming an additional decrease in Maillard browning at higher $a_w$ and moisture content (results not shown). Here, this decrease in Maillard browning occurred in parallel with a decrease in acrylamide formation (**Figure 5**). For lower $a_w$ (<0.9) and moisture content levels, however, these two phenomena are not related. Although a maximum browning at intermediate $a_w$ was observed (**Figure 2**), the final acrylamide concentration remained constant between 0.14 and 0.8 $a_w$ (**Figures 3** and **4**). However, the molar acrylamide yields of the heated mixtures correlated linearly with the relative absorbance values ($r^2$ > 0.93) for all investigated moisture contents. These correlations were however only observed when samples were compared with the same initial $a_w$ but not between mixtures of different $a_w$ or moisture content. Earlier studies (*14*, *36*, *37*) also suggest that, in some systems, color may be a good marker of the acrylamide content but the two are not implicitly linked. Consequently, the sample color cannot unambiguously predict the acrylamide content.

In contrast to our findings, Elmore et al. (*38*) demonstrated that acrylamide formation only occurred to any extent when final moisture levels in potato, rye, and wheat cakes had fallen below 5% (w/w). Below 5% moisture, acrylamide formation was inversely proportional to the moisture content. However, this significant increase in acrylamide formation was also caused by a more prolonged heat treatment, resulting in lower final moisture contents of the prepared products. Probably, this steep increase was also linked to this extra thermal input, in connection with physicochemical transformations occurring simultaneously (*7*). Moreover, because water evaporation takes place during the initial phase of heating, the inner temperature of the product did not exceed 100 °C until all free water was evaporated. Because acrylamide is not generated at temperatures below 100 °C, it is obvious that a significant increase in formation was only observed when all free water was evaporated, coinciding with a dramatic increase of the inner product temperature. Consequently, the findings of Elmore et al. (*38*) are more likely linked to a different thermal input of the foodstuff upon heating, rather than to a different moisture content. In our closed model system, however, the parameter water evaporation was eliminated. Consequently, both the thermal input and moisture content were kept more constant upon the entire heating duration, allowing for an assessment of the actual impact of moisture content and revealing no significant acrylamide increase upon decreasing the moisture content to about 4%, the lowest point tested.

Sadd and Hamlet (*14*) performed experiments in a closed dough model system also with a constant thermal input and found gradually decreasing acrylamide concentrations upon increasing the moisture content. Four moisture contents were tested: 2.7, 10, 19, and 45%. The water activity was however not measured. On the basis of sorption isotherm studies on cereal products (*31*, *35*), it is likely that these moisture contents correspond to a broad $a_w$ range, roughly between 0.1 and 0.9. Consequently, it could be stated that the final acrylamide concentrations in this dough model system decreased gradually upon increasing $a_w$ and the moisture content. A dilution effect of the acrylamide precursors for increasing the moisture content could cause this effect. For cereal products, the dilution of free asparagine in the free aqueous phase could be a controlling mechanism because free asparagine is the rate-limiting factor in these products (*37*). The difference in moisture content could also lead to changed kinetics of acrylamide formation. At very low-moisture contents, no decrease in acrylamide formation could however be demonstrated in this dough model system.

In potato products, however, reducing sugars are the rate-limiting factor. A similar dilution effect and changing kinetics could be expected upon increasing the moisture content and $a_w$. This was indeed demonstrated very recently by the kinetic study of Amrein et al. (*16*), where potato powders with different water content were heated inside a closed reactor system. An optimum rate of acrylamide formation was found at 13.3 g/100 g of dry matter. On the basis of the developed sorption isotherms, this corresponds roughly to 0.7 $a_w$. In addition, increasing activation energies for acrylamide formation at low-moisture contents (<25 g/100 g of dry matter) were demonstrated.

Our potato model system however did not reveal a significant decrease in molar acrylamide yields at the lowest moisture contents investigated. From previous studies (*14*, *16*) and above-mentioned results, it appears that low-moisture contents lead to a leveling off or a decrease in acrylamide formation, depending upon the lowest moisture content investigated and the experimental setup. Anyhow, it could be stated that at least a certain amount of water is needed for acrylamide formation to occur upon thermal treatment (*15*).

In accordance with the Amrein study (*16*), however, the above-mentioned results show an optimum molar yield of acrylamide (**Figure 5**). However, this optimum was situated at a higher moisture content of about 100% (on a dry basis) for the mixtures heated for 5 and 7 min at 170 °C. Nevertheless, this optimum was not observed for the mixtures heated for 3 min, indicating that a different (lower) thermal input into the model system may also play a role in the kinetics and thus the final molar acrylamide yield, for the different moisture contents. The different optimum of acrylamide yield between the Amrein study (*16*) and above-mentioned results may also be attributed to this different thermal input in the different model systems used.

Upon deep frying of potato strips, the moisture content decreases from 400 to about 30−80 g/100 g of dry matter, depending upon the oil temperature (*8*). Frying of potato slices even causes a more pronounced drop of the final product moisture content (*7*). According to the results presented in **Figures 3**−**5**, there is a phase throughout the deep-frying process of both products during which the acrylamide formation may be stimulated when the product moisture content passes 100% (on a dry basis). As shown in **Figures 3**−**5** for the mixtures heated for only 3 min, a different (lower) thermal input may however have a decreasing impact on the final acrylamide yield and thus acrylamide kinetics, as also suggested previously (*16*). However, when this reducing measure is applied in real foodstuffs, other aspects such as product quality characteristics should then also be safeguarded.

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