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Formation of Maillard Reaction Products during Heat Treatment of Carrots

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ABSTRACT: As indicators of the early stage of the Maillard reaction in carrots, *N*-(furoylmethyl) amino acids (FMAAs) formed during acid hydrolysis of the corresponding Amadori products were analyzed using RP-HPLC with UV detection. N^{ε}-FM-Lys (furosine), FM-Gly, FM-Ala, FM-Val, FM-Ile, FM-Leu, and FM-GABA were identified using synthesized standard material by means of mass spectrometry. Furthermore, N^{ε}-carboxymethyllysine (CML) and pyrraline were analyzed as indicators for advanced stages of glycation. For commercial samples with high water content, the formation of Amadori compounds predominates, whereas the advanced stage of Maillard reaction plays only a minor part. Carrot juices, baby food, and tinned carrots showed quite low rates of amino acid modification up to 5%. For dehydrated carrots, significantly higher values for Amadori products were measured, corresponding to a lysine derivatization of up to 58% and nearly 100% derivatization of GABA. Drying experiments revealed great differences in reactivity between the amino acids studied. Whereas furosine reached constant values quite quickly, some FMAAs showed a continuous increase with heating time, indicating that selected FMAAs can be used as a hallmark for the early Maillard reaction to control processing conditions.

KEYWORDS: carrots, Maillard reaction, furosine, furoylmethyl amino acid (FMAA), pyrraline, N^{ε}-carboxymethyllysine (CML)

INTRODUCTION

Processing of foods leads to modifications of ingredients. Especially during thermal treatment and storage, the Maillard reaction, also referred to as nonenzymatic browning or glycation, is of particular importance for food browning and the formation of aroma and flavor substances.[1,2] Besides desired sensory changes, the Maillard reaction also results in an irreversible blockage of essential amino acids and consequently a decrease in the nutritional quality of food proteins.[3]

During the early stage of the Maillard reaction, Amadori products (N-substituted 1-amino-1-deoxy-2-ketoses, APs) are formed by the reaction of aldoses with free amino groups from amino acids, peptides, and proteins.[2] Because Amadori products generally arise before sensory changes become noticeable, they are used as early indicators for quality changes caused by glycation reactions.[4] For quantification, Amadori compounds can be converted into *N*-(2-furoylmethyl) amino acids (FMAAs) by acid hydrolysis, which are measurable by RP-HPLC with UV detection.[5−7] Besides furosine, which results from derivatization of the ε-amino group of protein-bound lysine, also free amino groups from the N-termini of peptides or proteins as well as free amino acids are modified.

Amadori compounds are degraded to highly reactive α-dicarbonyls, such as glyoxal, methylglyoxal, and 3-deoxyglucosulose, during the advanced stage of glycation. Reactions of these α-dicarbonyl compounds with lysine and arginine (free or side chains in proteins) lead to stable endproducts, the so-called advanced glycation endproducts (AGEs).[4] Important lysine derivatives in foods are N^{ε}-carboxymethyllysine (CML)[8,9] and pyrraline,[10] whereas pentosidine is a cross-link product between lysine and arginine.[11,12]

Amadori compounds and FMAAs have been identified in fruits and vegetables, for example, in orange juice,[13−15] processed tomato products,[15,16] and dehydrated fruits.[17] Reutter and Eichner determined free Amadori compounds in air-dried carrots and other vegetables directly by RP-HPLC with postcolumn derivatization.[18] Furosine and different α-FMAAs were detected in dehydrated carrots by Soria et al.[19] However, less is known about the extent of the advanced stage of Maillard reaction in carrots.

The aim of this study, therefore, was to determine the contents of different FMAAs, pyrraline, and N^{ε}-carboxymethyllysine in commercial carrot products and to investigate the formation of these Maillard reaction products (MRPs) during heating and drying of carrots.

MATERIALS AND METHODS

Chemicals and Reference Compounds. Methanol was purchased from VWR (Darmstadt, Germany). Amino acids, caffeine, methanol for CLND measurement, and pepsin and Pronase E for enzymatic hydrolysis were obtained from Merck (Darmstadt, Germany). Aminopeptidase M and prolidase for enzymatic hydrolysis were from Sigma-Aldrich (Taufkirchen, Germany). Furosine dihydrochloride was purchased from Neo MPS (Strasbourg, France). All chemicals were of the highest purity available. Reference material for pyrraline was synthesized according to the method of Henle and Bachmann.[10] Reference materials for CML and CMO were synthesized according to the method of Delatour et al.[24]

Materials. Commercial samples of different carrot products ($n = 15$) were purchased in local stores and consisted of one raw sample, five samples of carrot juices (one containing 3% honey, one with lemon juice), four samples of baby food, two samples of tinned carrots, one sample of carrot salad, and two samples of dehydrated carrots.

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Preparation of FMAAs for Identification. *N*-(1-Desoxy-D-fructosyl) amino acids (Fru-AAs) were obtained according to ref 18. Briefly, 2 mmol of each amino acid (alanine, leucine, isoleucine, valine, glycine, γ-aminobutyric acid) and 12 mmol of glucose were dissolved in 84 mL of methanol and heated at 90 °C under reflux for up to 8 h. *N*-(1-Desoxy-D-fructosyl) glycine was prepared similarly with the addition of 2 mmol of sodium metabisulfite.[20] Solvent was removed under vacuum, and the residues were dissolved in 10 mL of distilled water and filtered (0.45 μm). For conversion of Fru-AAs into FMAAs, 500 μL of each Fru-AA solution was hydrolyzed after the addition of 1 mL of 12 M hydrochloric acid at 110 °C for 23 h. The hydrolysates were filtered and dried under vacuum. For analysis, the dried residues were dissolved in 0.2 M hydrochloric acid, followed by membrane filtration (0.2 μm). The identity of the corresponding FMAA was confirmed by LC-ESI-TOF-MS.

Heating and Storage Experiments. All experiments were performed with carrots of the variety Laguna. Juice was separated from fresh peeled carrots. Aliquots of the fresh juice (10 mL) were heated at 90, 100, and 120 °C for 10, 20, and 30 min. Before analysis, samples were lyophilized. Fresh peeled carrots were sliced (thickness = 2 mm). Aliquots (five slices) were dried in a drying oven at 70, 80, and 90 °C for 30 min and 1, 2, 3, and 5 h. The nitrogen contents of all investigated carrot samples were measured by using the Kjeldahl method, and protein contents were calculated using the specific factor of 6.25.

Identification of FMAAs by LC-ESI-TOF-MS. For identification of FMAAs, an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA) was used, consisting of a high-pressure gradient pump system, column oven, automatic injector, and diode array detector, combined with a PerSeptive Biosystems Mariner time-of-flight mass spectrometer (TOF-MS) equipped with an electrospray ionization source (ESI) (Applied Biosystems, Stafford, CA). Ionization was carried out in positive mode. RP-HPLC was accomplished as described for quantification of FM-Val, FM-Leu, and FM-Ile. The following MS conditions were used: quadrupole RF voltage, 799.80; nozzle temperature, 140.01 °C; reflector potential, 1549.99; detector voltage, 2299.99; first mass, 100; last mass, 700.[21] The *m*/*z* values of the molecular ions $(M + H)^+$ of the FMAAs were as follows: furosine, *m*/*z* 255; FM-Gly, *m*/*z* 184; FM-Ala, *m*/*z* 198; FM-GABA, *m*/*z* 212; FM-Val, *m*/*z* 226; FM-Ile, *m*/*z* 240; FM-Leu, *m*/*z* 240.

Determination of Conversion Factors (Degradation of Fru-AAs to FMAAs). Conversion factors were determined according to the method of Penndorf et al.,[21] using RP-HPLC with chemiluminescence nitrogen detection (CLND). For this, an Agilent 1100 series HPLC system (Agilent Technologies) composed of a high-pressure gradient pump system, column oven, automatic injector, and UV detector was used in combination with a nitrogen-sensitive detector Antek 8060 (Baumel, Houston, TX). For chromatographic separation a Eurospher 100-5 C18 column (5 μm, 250 × 3 mm, Knauer, Berlin, Germany) was used at a flow rate of 0.2 mL/min and a temperature of 30 °C. Ten microliters of each Fru-AA and FMAA solution was analyzed using water (solvent A) and methanol (solvent B), each containing 2% of formic acid, and the following gradient: 0% solvent B for 5 min, to 5% B in 15 min, to 80% B in 10 min, held at 80% B for 5 min, to 0% B in 5 min, and held at 0% B for 20 min. For external calibration, a solution of caffeine (2 mg/mL) was used.

Quantification of FMAAs. For quantification of FMAAs in carrot samples, acid hydrolysis was accomplished with 8 M hydrochloric acid at 110 °C for 23 h. FM-Val, FM-Leu, and FM-Ile were quantified in the carrot samples according to ref 21. Hydrolysates were dried under vacuum, dissolved in 0.2 M hydrochloric acid, and filtered (0.2 μm). For determination, a HPLC system (Knauer) was used consisting of a WellChrom pump K-1001, a gradient mixing system, a diode array detector WellChrom K-2700, a column oven, and an automatic injector Marathon. For separation a Eurospher 100-5 C18 column (5 μm, 250 × 4.6 mm, Knauer) with precolumn was used at a flow rate of 0.7 mL/min and a temperature of 25 °C. The following gradient with water (solvent A) and methanol (solvent B), each containing 2% formic acid, was applied: 0% solvent B for 10 min, to 10% B in 5 min, to 20% B in 30 min, to 80% B in 5 min, held at 80% B for 5 min, to 0% B in 5 min, and held at 0% B for 10 min. Detection wavelength was 280 nm.

For determination of furosine, FM-Ala, FM-GABA, and FM-Gly, hydrolysates were cleaned up using C18 material (C18-Max, Grace Davison Discovery Sciences, delivered by Alltech Grom, Rottenburg-Hailfingen, Germany). FMAAs were eluted with 3 mL of 3 M hydrochloric acid, dried under vacuum, dissolved in 0.2 M hydrochloric acid, and filtered. Analysis was carried out by ion-pair chromatography according to ref 7 using the HPLC system described above and an RP8 column "furosine dedicated" (Grace Davison Discovery Sciences/Alltech, Sedriano Mi, Italy). For separation a gradient with water containing 0.4% acetic acid (solvent A) and water containing 0.4% acetic acid and 0.3% KCl (solvent B) was used as follows: 0% solvent B for 15.5 min, to 50% B in 7 min, held at 50% B for 2.5 min, to 0% B in 2 min, and held at 0% B for 7 min. The flow rate was 1.2 mL/min, and a temperature of 33 °C was applied. For external calibration of all FMAAs, furosine (Neosystem, Strasbourg, France) was used.

Quantification of Pyrraline. Pyrraline was quantified after a three-step enzymatic hydrolysis with pepsin, Pronase E, aminopeptidase M, and prolidase. Determination by RP-HPLC/UV was accomplished according to the method of Foerster et al.,[22] using the HPLC system described for the determination of FMAAs and a Eurospher 100-5 C18 column (5 μm, 125 × 4.6 mm, Knauer).

Quantification of N^{ε}-Carboxymethyllysine (CML). CML was determined after acid hydrolysis under nitrogen to avoid degradation of ε-Fru-Lys and cleanup at C18 and cation exchange material (C18-Max and Cation-X, Grace Davison Discovery Sciences, delivered by Alltech Grom, Rottenburg-Hailfingen, Germany). As internal standard, N^{ε}-carboxymethylornithine (CMO) was added. CML and CMO were analyzed as trifluoroacetyl methyl esters by single-ion monitoring GC-MS (SIM-GC-MS) according to ref 23. A GC-MS system (Agilent Technologies) was utilized consisting of a GC system 6890 series, autosampler, injector 7683 series, and mass selective detector 5973 series. The column was a Zebron ZB-5 (Phenomenex, Aschaffenburg, Germany). The 392 (CML) and 351 (CMO) ions were used for quantification.

Determination of Amino Acids. For calculation of amino acid blockage, the amino acid composition was analyzed after acid hydrolysis (6 M hydrochloric acid, 110 °C, 23 h) by ion-exchange chromatography and postcolumn derivatization with ninhydrin according to ref 25. An amino acid analyzer S4300 (SYKAM, Fürstenfeldbruck, Germany) was used. Free amino acids were determined after centrifugal ultrafiltration of the water extracts (10 g of carrot sample in 10 mL of water) with Vivaspin 4 concentrators (MWCO 5000 Da, Sartorius, Göttingen, Germany).

RESULTS AND DISCUSSION

Identification and Quantification of FMAAs. Reference samples containing the furoylmethyl derivatives of glycine, alanine, γ-aminobutyric acid (GABA), valine, leucine, and isoleucine were prepared, and the identity was confirmed by the presence of the specific molecular ion $(M + H)^+$. These samples were used as reference for identification of the FMAAs in carrot samples. Two different separation methods were used. Furosine, FM-Gly, FM-Ala, and FM-GABA were determined by using an RP8 column, whereas for quantification of the more hydrophobic derivatives (FM-Val, FM-Leu, and FM-Ile), an RP18 column was applied. FMAAs were identified by comparison of retention times with reference samples and the characteristic UV spectrum

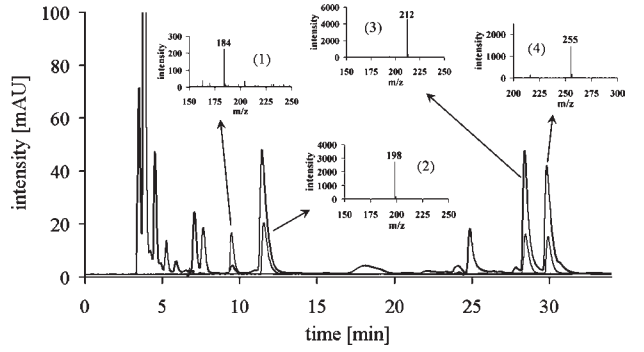

Figure 1. RP-HPLC chromatogram (λ = 280 nm) for determination of (4) furosine, (1) FM-Gly, (2) FM-Ala, and (3) FM-GABA in a dried carrot sample (black) and reference substances (gray). Mass spectra show specific molecular ions $(M + H)^+$.

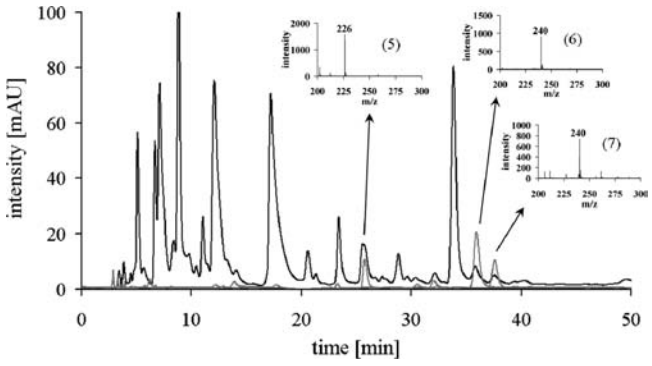

Figure 2. RP-HPLC chromatogram (λ = 280 nm) for determination of (5) FM-Val, (6) FM-Ile, and (7) FM-Leu in a dried carrot sample (black) and reference substances (gray). Mass spectra show specific molecular ions $(M + H)^+$.

(absorption maximum at 280 nm). The identity was verified by the specific molecular ion obtained via LC-MS. The chromatograms of a dried carrot sample and the mass spectra of the identified FMAAs are shown in Figures 1 and 2. Each of the investigated FMAAs could be detected in this sample. The detection limit of the RP-HPLC-DAD methods for FMAAs was 10 mg/kg protein, and the determination limit was 30 mg/kg protein. Relative standard deviations were between 1 and 5%, measuring the commercial samples as triplicates and the samples of heating experiments as duplicates.

Furosine, FM-GABA, and FM-Ala have already been identified in dehydrated carrots by Soria et al.[19] The identification of the FM derivatives of glycine, valine, leucine, and isoleucine has not yet been described for processed fruits and vegetables. Whereas ε-Fru-Lys is predominantly formed by glycation of protein-bound lysine residues, the other amino acids can only be glycated when existing in free form or as N-terminal amino acid of peptides and proteins. GABA is a nonproteinogenic amino acid formed by decarboxylation of glutamate and exists in unbound form.[26]

Conversion Factors (Reaction of Fru-AAs to FMAAs). For calculation of the Amadori product (AP) contents and for estimation of the resulting degree of amino acid blockage, it was necessary to measure the relative amount of FMAA formed during acid hydrolysis of individual APs. This conversion rate

Table 1. Relative Molar Yields of FMAAs (Mean ± Standard Deviation Obtained from Duplicates) Obtained during Acid Hydrolysis of Amadori Compounds with 8 M HCl for 23 h at 110 °C and Resulting Conversion Factors

AP	yield of FMAA (%)	conversion factor
Fru-GABA	27.2 ± 0.2	3.7
Fru-Gly	8.5 ± 0.0	11.8
Fru-Ala	17.0 ± 0.3	5.9
Fru-Val	14.2 ± 0.0	7.0
Fru-Ile	13.8 ± 0.2	7.2
Fru-Leu	15.2 ± 0.1	6.6
ε-Fru-Lys	46.1 ± 1.9	2.2[a]

[a] Conversion factor for ε-Fru-Lys is according to Krause et al.[6]

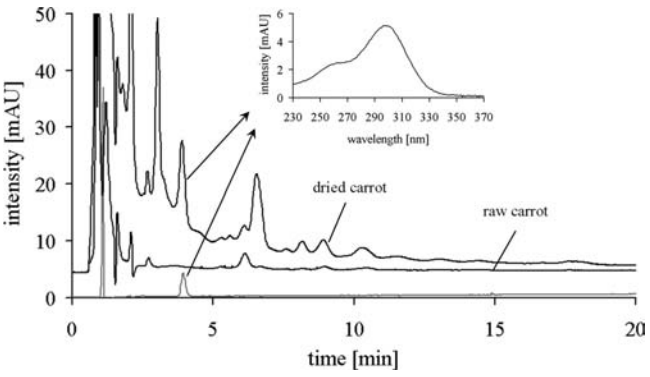

Figure 3. RP-HPLC chromatogram (λ = 297 nm) for the determination of pyrraline in a commercial dehydrated carrot sample (black) and reference substance (gray). UV spectrum shows specific UV absorption.

depends on the hydrolysis conditions (concentration of hydrochloric acid, temperature, and time) and the respective amino acid and reducing sugar. Whereas Krause et al. have investigated the molar yields of different hydrolysis products of ε-amino-modified lysine depending on acid concentration and different sugars,[6] less is known about the fructose derivatives of the other amino acids. Penndorf et al.[21] studied lactulosyl derivatives, but it is possible that fructose derivatives show different behavior. For determination of the conversion factors, contents of APs in the synthesis samples and respective FMAAs present in the hydrolysates after hydrolysis with 8 M HCl at 110 °C for 23 h were measured. For this, we used a HPLC system with a chemoluminescence nitrogen detector (CNLD) according to the method of Penndorf et al.[21] Table 1 shows the conversion factors determined. The data indicate that between 8.5 and 27.2% of the APs are transformed to the corresponding FMAAs, resulting in conversion factors between 3.7 and 11.8.

For quantification of the AP contents in carrot samples, the measured FMAA contents in millimoles per 100 g of protein were multiplied by the respective conversion factor. For estimation of amino acid blockage due to AP formation, the AP contents were compared with the amount of the corresponding amino acid, resulting from measurement of the intact amino acid by amino acid analysis.

MRPs in Commercial Carrot Samples. Besides APs formed during the early stage of the Maillard reaction, also the AGEs pyrraline and CML were determined. Identification of pyrraline

Table 2. Contents of FMAAs (after Acid Hydrolysis) and AGEs As Determined in Commercial Carrot Samples[a]

	n	FMAAs (mg/kg protein) of							AGEs (mg/kg protein)	
		N^{ε}-lysine	alanine	GABA	valine	leucine	isoleucine	glycine	pyrraline	CML
raw	1	nd	nd	nd	nd	nd	nd	nd	nd	nd
salad	1	297 (9)	nd	nd	13 (1.2)	nd	nd	nd	nd	47 (1.1)
juices	5	295–1763 (22–84)	61–257 (2–13)	73–436 (7–41)	44–285 (4–37)	73–333 (10–55)	nd–35 (nd–7.1)	nd	nd–134 (nd–4.2)	51–132 (1.8–3.8)
baby food	4	287–578 (14–29)	47–104 (2.3–3.7)	58–107 (8–11)	100–541 (10–49)	59–78 (5.1–6.8)	nd–114 (nd–16)	nd	51–96 (1.0–2.2)	97–148 (2.2–4.2)
tinned	2	1272–1553 (33–47)	nd–50 (nd–4.3)	nd	144–173 (10–11)	72–84 (4.0–4.3)	nd	nd	nd–158 (nd–2.2)	105–108 (1.7–2.0)
dried	2	15408–15529 (519–582)	6979–11368 (354–496)	6912–9123 (936–1000)	1505–2000 (188–239)	409–519 (48–56)	572–866 (102–151)	408–480 (42–50)	375–378 (5.8–6.4)	184–308 (3.9–5.9)

[a] In parentheses are given the data for resulting amino acid blockage due to formation of Fru-AAs in mmol/mol amino acid (italicized). *n*, number of samples; nd, not detectable (below 30 mg/kg protein).

has not yet been described for processed carrot products. Figure 3 shows the RP-HPLC chromatogram of a commercial dehydrated carrot sample. Pyrraline was identified by comparison of retention time, the characteristic UV spectrum (absorption maximum at 297 nm), and standard addition. Table 2 shows the contents of FMAAs and AGEs of a number of commercially available carrot products and the resulting rates of amino acid blockage due to these MRPs. It has to be noted that the calculated AP contents might be affected by some kind of error, because the used conversion factors refer to putative fructosyl derivatives. In complex matrices such as carrots also derivatives with other reducing sugars could be formed, which might show differing conversion rates. Alabran et al.[27] investigated the sugar composition of fresh carrots. Besides sucrose, glucose, fructose, and corresponding phosphates, 4.4% of total sugar remained unknown. These could slightly involve other mono- and disaccharides or higher oligosaccharides.

In raw carrots no or only negligible amounts of Maillard reaction products were detected. For the carrot salad sample, 1% lysine blockage due to ε-Fru-Lys was found. However, each of the investigated APs, except Fru-Gly, could be identified in the juices, carrot-containing baby foods, and tinned carrots. Low rates of amino acid blockage of up to 5% were calculated in the majority of the samples. The samples of carrot juices and of the carrot-containing baby foods did not differ substantially concerning the contents of Maillard reaction products. In particular, for the tinned carrots, higher amounts of ε-Fru-Lys up to 47 mmol/mol Lys were measured, which is probably due to the sterilization processes. The advanced glycation compounds pyrraline and CML contribute to lysine blockage with up to 0.4%.

Concentrations of all investigated Maillard reaction products strongly increase in dehydrated carrot samples, and considerably high degrees of amino acid blocking were observed. Furthermore, Fru-Gly, which was not detectable in any other commercial samples, could be identified. The contents of ε-Fru-Lys in two samples were 15.4 and 15.5 g/kg protein, corresponding to a lysine blockage of up to 58%. Lysine derivatization by the advanced stage products pyrraline and CML were up to 0.6%, respectively. High blockage rates up to 50% were also noted for alanine. For GABA a nearly complete derivatization to the Amadori product was found. Moderate losses of valine up to 24% and of isoleucine to 15% were measured, whereas leucine and glycine seemed to be less reactive with blockage rates of around 5%.

The quantitative data of some FMAAs obtained by Soria et al. for two commercial samples of dehydrated carrots were 3.58 and 4.16 g for furosine, 1.52 and 3.12 g for FM-GABA, and 1.34 and 1.54 g for FM-Ala per kilogram of protein, respectively.[19] Compared to these data, the amounts found in our study are 3−7-fold higher, probably due to more intense heating conditions applied during drying. The formation of CML and pyrraline has been investigated predominantly in milk products, infant formula, and bakery products,[28−32] whereas data about heat-treated fruit and vegetable products have not yet been reported. Contents in the dehydrated carrot samples were in the dimension of those measured for ultrahigh-temperature and sterilized milk.

Heated Carrot Juices. For investigation of MRP formation during heat treatment, heating experiments were accomplished. For this, juice separated from raw carrots of one variety was heated for 10−30 min at 90−120 °C. Heat treatment of fresh carrot juice caused only a marginal formation of early Maillard reaction products. Whereas FM-Ala, FM-Ile, FM-Leu, and FM-

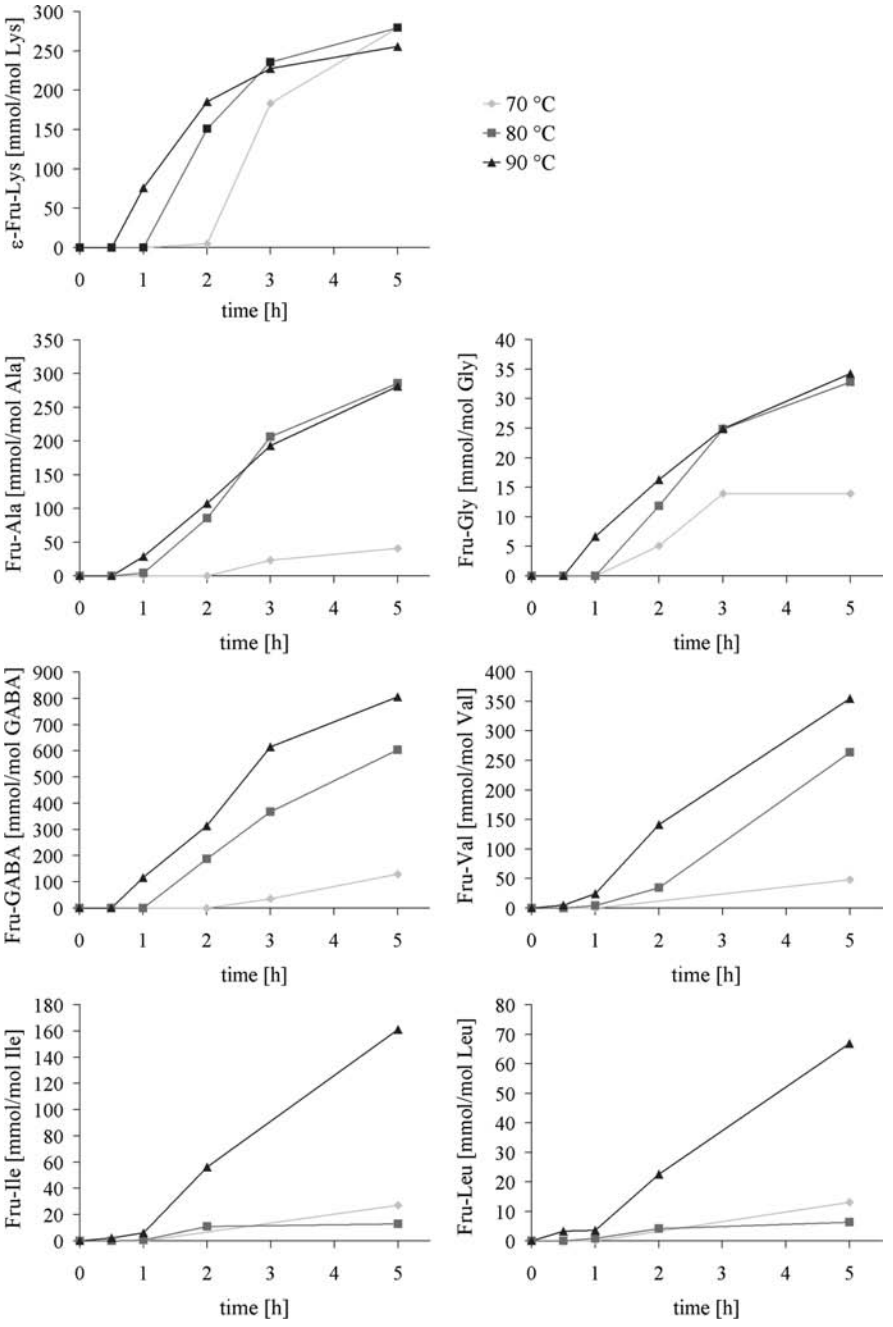

Figure 4. Formation of Fru-AAs during drying of carrots.

Gly were not detectable in the hydrolysates of heated samples, an increase of furosine, FM-GABA, and FM-Val was observed during heating at 120 °C for 30 min. Thereby, 1.8% of lysine and 0.5% of GABA and valine, respectively, were modified to APs. CML was not detectable, whereas pyrraline could be quantified in the juice sample heated at 100 °C for 20 min at a concentration of 2.1 mmol/mol Lys. With more drastic heating conditions, the pyrraline content increased to 3.5 mmol/mol Lys (120 °C for 30 min). Taken together, the extent of Maillard reaction during the heating experiments was lower than in commercial juice samples. This is probably due to differences in the accomplishment of heat treatment in the food-processing industry and the model heating experiments performed in this study. Industrial production of carrot juice is composed of several heating steps, including steam-peeling and blanching of the carrots, followed by grinding and heating of the mash for enhanced yield of juice. Finally, the extracted juice has to be sterilized. Every heating step contributes to the formation of the final amount of Maillard products. The sensitive quantification of FMAAs, therefore, can be a tool to identify the individual contribution of each step of carrot technology to obtain minimally processed products.

Dried Carrots. Because considerably high degrees of amino acid blockage caused by AP formation were observed in commercially dehydrated carrots, drying experiments with carrots were performed to investigate the reactivity of the respective

amino acids. For this purpose, slices of fresh carrots of the same variety were dried at different temperatures (70−90 °C) for different times (30 min−5 h), and the MRPs were quantified.

All investigated FMAAs could be detected in the dehydrated carrots, and increasing contents were observed with higher temperatures and longer times of drying. Figure 4 shows the courses of formation for the investigated APs, which reveal differences in the reactivity of the respective amino acids. Independent of the temperature, a nearly constant lysine blockage by ε-Fru-Lys of 28% was observed after 5 h. The curves of Fru-Ala and Fru-Gly formation were similar, and plateaus were reached after 5 h at 80 and 90 °C, whereas the concentrations were much lower after 5 h at 70 °C. Although derivatization of total glycine (3%) was much lower than that of alanine (29%), blockage rates of the respective free amino acids were comparable (53%). In contrast, only low amounts of Fru-GABA and Fru-Val were formed at 70 °C, but the concentrations rose with higher temperatures and heating times. The maximum blockage rate of GABA, which only occurs free, was 81%, whereas 35% of total and 56% of free valine were derived by the AP. Leucine and isoleucine seem to be less reactive toward glycation, as considerable increases of the amounts of Fru-Leu and Fru-Ile were observed only for samples heated at 90 °C and derivatization rates of the respective free amino acids were lower than for other APs (30% for Ile and 24% for Leu).

Taken together, amino acid blockages between 3 and 81% were reached. Thus, when compared to heat treatment of juices, drying of carrots generated 30−60-fold higher amounts of early-stage MRPs. Only very small amounts of reaction products resulting from advanced stages of glycation reactions were formed. It was not possible to unambiguously identify pyrraline in the samples. CML was not formed during heating at 70 and 80 °C, but at 90 °C the content increased to 2.1 mmol/mol Lys, which is comparable to that in the commercial samples.

In conclusion it can be summarized that during heat treatment of carrots, the formation of Amadori compounds predominates, whereas the advanced stage of Maillard reaction plays only a minor part. Besides ε-Fru-Lys, Amadori products arising from free amino acids are highly relevant. Whereas furosine is commonly used as a marker for the thermal treatment of foods, for carrot products the FMAAs are more suitable indicators. In particular, FM-GABA and FM-Val seem to be suitable heat markers, as they show a continuous increase with rising temperature and duration of heat treatment, whereas the content of furosine reaches a constant level due to further reactions of ε-Fru-Lys.

Furthermore, it must be noted that the extent of the early Maillard reaction expressed as blockage of amino acids is low for heat-treated carrot products with high water contents such as juices. For such samples the Maillard reaction does not have a significant impact on the nutritional quality of the final product. Drying, however, leads to significantly higher rates of amino acid blockage. Our studies point to the fact that certain amino acids may exist predominantly as Amadori products in dried carrot products. The physiological consequences of this phenomenon are unknown. According to Finot et al.,[5] Amadori products of lysine are not available as lysine during digestion. It is highly likely that the same is due for Amadori products of other amino acids. Although carrots usually do not play an important role as a dietary source for amino acids in human nutrition, technological processes should be designed in a way that their negative impact on the nutritional value of the final product is as little as possible. The analytical strategies shown in this paper may contribute to a better control of corresponding processes.

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DISCLOSURE

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ABBREVIATIONS USED

FMAA, furoylmethyl amino acid; Fru-AA, fructosyl amino acid; MRP, Maillard reaction product; AGE, advanced glycation endproduct; CML, N^{ε}-carboxymethyllysine; ESI, electrospray ionization; TOF-MS, time-of-flight mass spectrometer; CLND, chemiluminescent nitrogen detector.

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