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Effect of heat treatment and dough formulation on the formation of Maillard reaction products in fine bakery products – benefits and weak points

ZUZANA CIESAROVÁ – KRISTÍNA KUKUROVÁ – ALENA BEDNÁRIKOVÁ – FRANCISCO J. MORALES

Summary
Possibly harmful compounds (acrylamide, 5-(hydroxymethyl)-2-furfural – HMF), markers of the Maillard reaction extent (furosine, fluorescence, browning, colour), as well as beneficial radical-scavenging capacity were determined in fried fine bakery products, rosquillas, at different heat treatment and dough formulation. In different simplified recipes, saccharose was substituted by glucose and fructose, and a raising agent was added while different temperature and time regimes were applied. Duration of frying from 4 min to 8 min increased the acrylamide content from (43 ± 8) $\mu g \cdot kg^{-1}$ to (159 ± 12) $\mu g \cdot kg^{-1}$ at 180 °C, and from (94 ± 3) $\mu g \cdot kg^{-1}$ to (366 ± 5) $\mu g \cdot kg^{-1}$ at 200 °C. Sodium hydrogen carbonate addition resulted in an only weak (13%) decrease in the acrylamide content, but in a more pronounced (80%) suppression of HMF formation. Substitution of saccharose for the mixture of glucose and fructose caused a decrease in acrylamide contents instead of its expected increase, but HMF formation was strongly supported by the presence of fructose. On the other hand, beneficial properties, such as radical-scavenging capacity, browning, colour, and fluorescence parameters were developing with the advance of Maillard reaction. Colour parameters were in good correlation with radical-scavenging capacities of final products prepared from saccharose (correlation coefficients, 0.779–0.981) as well as with acrylamide contents in samples made under all recipe modifications (correlation coefficients, 0.882–0.979).

Keywords:
acrylamide; hydroxymethylfurfural; asparagine; furosine; bakery products; Maillard reaction; browning

Thermal treatment, a common way of improving digestibility, safety and quality of many foods, is used for ages. Besides unambiguous desirable aspects of Maillard reaction associated with this treatment, which is responsible for the development of the brown colour and many sensorially active components of food, certain detrimental effects of this process are still emerging, e.g. loss of nutritionally important compounds and undesirable generation of contaminants. Some Maillard reaction products (MRP) such as acrylamide, heterocyclic amines, β-carboline, furan, hydroxymethylfurfural, are related to a variety of diseases, e. g. diabetes and cancer [1]. However, the hazard of human carcinogenesis caused by the consumption of MRP seems to be relatively small. In contrast, many positive effects have been attributed to MRP, such as antioxidant activity originating in particular in melanoidins and pronyl-lysine present in the bread crust, which have been shown to have beneficial effects on human health [1]. The antioxidant activity of MRP also contributes substantially to the shelf life of heat-treated food products.

Among the mentioned harmful aspects, the acrylamide issue has taken great attention since 2002 due to its frequent occurrence in food products processed at temperatures higher than 120 °C [2]. The recent assessment by the Joint FAO/WHO Expert Committee on Food Additives since 2005 has confirmed that a risk following from the estimated exposure to acrylamide by dietary intake cannot be excluded. Acrylamide is classified as a probable human carcinogen by the International Agency for Research on Cancer [3, 4]. Moreover, with respect to the last observations confirming the association between acrylamide intake and endometrial, ovarian, renal, and breast cancer risks [5–7], the demand for acrylamide mitigation seems urgent.

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Initial data on acrylamide content in food products indicated that carbohydrate-rich foods, in general, have higher acrylamide contents [2]. Several researchers established that the main pathway of acrylamide formation in foods is linked to the Maillard reaction and, in particular, to reactions of the amino acid asparagine [8, 9]. The link of acrylamide to asparagine, which directly provides the backbone of the acrylamide molecule, was confirmed by labelling experiments [9, 10]. Studies to date clearly show that the amino acid asparagine is mainly responsible for acrylamide formation in heated foods after a condensation of its amino group with reducing saccharides or a carbonyl source. Moreover, the saccharide-asparagine adduct, *N*-glycosylasparagine, generates high amounts of acrylamide, suggesting the early Maillard reaction as a major source of acrylamide [9]. In addition, decarboxylated asparagine (3-aminopropionamid), when heated, can generate acrylamide in the absence of reducing saccharides [10]. A good evidence supporting the early Maillard reaction as a main pathway involving early decarboxylation of the Schiff base, rearrangement to the resulting Amadori product, and subsequent beta-elimination to release acrylamide was presented by YAYLAYAN [11].

A further important aspect is that the saccharide type may affect the yield of acrylamide. Initial works indicate that *keto* saccharides, such as fructose, seem more efficient than *aldehydo* saccharides (e.g. glucose), generating acrylamide at relatively lower temperatures. A mechanistic explanation is the ability of the fructose Schiff intermediate to stabilize the azomethine ylide intermediate through H-bonding [12]. An alternative possibility is the higher reactivity of fructose due to its lower melting point and thus greater molecular mobility, thereby favouring a faster reaction with the amine to form the Schiff base [13].

The formation of acrylamide and related vinylogous compounds takes place only in a "dry" environment. In aqueous systems, the carboxylated Amadori compounds are considered as first stable intermediates formed in the Maillard cascade leading to 1- and 3-deoxysones, and that further decompose to form important colour and flavour compounds. A dry or low-moisture state with an adequate thermal input enables the decarboxylation of *N*-glycoside, opening the route to acrylamide [14, 15].

Overall, reaction yields of acrylamide in foods are difficult to predict because of its concurrent elimination [16]. The higher the temperature, the more acrylamide is formed, but also the faster is its elimination. The prediction and controlling of acrylamide formation in food are still under investigation in various model systems as well as real food matrices [17, 18].

Another one of the numerous compounds resulting from the heating of foods is 5-(hydroxymethyl)-2-furfural (HMF). It is formed by acid-catalysed dehydration of reducing saccharides in the Maillard reaction. It was found at high levels in numerous food products. Despite the fact that HMF does not seem to pose any significant toxicological problem, there is a number of structural alerts that point to a possible risk of genotoxic and carcinogenic activity of this compound [19]. HMF is an intermediate in the Maillard reaction, which occurs when reducing hexose moieties are heated in the presence of amino acids or proteins [20]. An alternative source of HMF involves direct thermal dehydration of fructose, saccharose and, to a lesser extent, glucose [21]. This reaction does not require the presence of amino groups. It is strongly enhanced under acid conditions.

Cereal-based foods are a part of the staple diet and encompass an extremely diverse range of products. Those of importance in relation to acrylamide and HMF occurrence include bread, bakery products, and breakfast cereals.

Based on ongoing efforts in a benefit versus risk approach to evaluate the impact of thermal treatment on processed foods, the objective of this study was to compare beneficial and harmful effects of this process in relation to thermal conditions and dough formulation of traditional Spanish fried fine bakery products, rosquillas. The modifications in dough formulation involved alterations in saccharides and raising agents, and frying conditions were varied. Contents of acrylamide, HMF, furosine as a marker of Maillard reaction early stage as well as colour development during processing through trichromatic (CIELab), fluorescence (FIC) and browning evaluation, respectively, were determined.

MATERIAL AND METHODS

Chemicals and standards

L-asparagine (Asn), *S*-(+)-aspartic acid (Asp), *S*-(+)-glutamine (Gln), *S*-(+)-glutamic acid (Glu), D-glucose (Glc), D-fructose (Fru), saccharose, sorbitol (Sigma, St. Louis, USA or Merck, Schuchardt, Germany); acrylamide, purity 99% (Sigma-Aldrich), *d3*-acrylamide (2,3,3-*d3*-2-propenamide), *d3*-glutamic acid (Cambridge, Isotope Laboratories, Maryland, USA), furosine (Neosystem Laboratories, Strasbourg, France), acetonitrile, methanol (HPLC-grade, Scharlau, Barcelona, Spain

or Sigma-Aldrich, Steinheim, Germany); perfluorooctanoic acid (PFOA) (Aldrich, Steinheim, Germany); acetic acid p.a. (Lachema, Brno, Czech Republic); $NaHCO_3$ (>99.5%, Panreac, Barcelona, Spain), deionized water; flour (Valpan artesanos – Santa Rita Harinas, Guadalajara, Spain); sunflower oil (Coosur, Sevilla, Spain).

Dough preparation and frying conditions

Dough was prepared according to the simplified recipe with 323 g of wheat flour, 92 g of saccharides (saccharose or equimolar mixture of glucose and fructose) and 185 ml of water. Sodium hydrogen carbonate was used in recipe 2 as a raising agent in an amount of 2.4 g. The recipes are listed in Tab. 1. All ingredients were thoroughly mixed by a lab-scale dough blender (Model HR1570; Philips, Eindhoven, Netherlands). In the next step, the dough was formed to rolls with a weight of (10.0 ± 0.5) g and a length of (10 ± 0.2) cm, and subsequently fried at 180 °C or 200 °C for 4, 6, and 8 min in sunflower oil using a fryer with a capacity of 3 l and 1200 W power (Taurus, Barcelona, Spain). The temperature of the fryer had been calibrated previously, with external thermocouples (type K, 0.1 mm) and the temperature profile was continuously recorded by a datalogger and evaluated for precision. After frying, samples were dried on paper, cooled to room temperature, grinded, put into containers and stored at –20 °C until analysis.

Tab. 1. Composition of dough prepared according to different recipes with determined pH values.

	Recipe 1	Recipe 2	Recipe 3
water [ml]	185	185	185
saccharose [g]	92	92	0
glucose [g]	0	0	46
fructose [g]	0	0	46
$NaHCO_3$ [g]	0	2.4	0
flour [g]	323	323	323
pH value	6.5	8.6	6.6

Moisture

The moisture content was determined by a gravimetric method as described in AOAC-925.10 [22]. Samples were homogenized in a household cutter Moulinette (Moulinex, Paris, France) and amounts of 2 g were weighed into Chopin dishes. They were dried in a convection oven Digiheat (JP-Selecta, Barcelona, Spain) till they reached a constant weight after three consecutive readings at (105 ± 1) °C. Analyses were carried out in duplicate.

Protein determination

Samples (0.800–1.000 g) were analysed by the AOAC 992.15 procedure [23] for a total protein content by heating to (105 ± 1) °C in a LECO model FP-2000 protein/nitrogen analyser (Leco Instruments, Madrid, Spain) calibrated with ethylendiamine tetraacetic acid (EDTA, Dumas method). The nitrogen-to-protein conversion factors used were 5.70 for wheat. Results were expressed as g of protein per 100 g of product.

Colour measurement

Colour of the grinded samples was evaluated as reflectance using a spectrophotometer CM-3500d (Konica Minolta, Osaka, Japan) according to the CIELab (the $L^*a^*b^*$ colour space also referred to as CIELab defined by CIE, Commission Internationale de l`Eclairage, the international organization concerned with light and colour). The system gives the values of three colour components: the luminosity L^* (–black to +white component) and the chromaticity coordinates, a^* (+red to –green component) and b^* (+yellow to –blue component). A powdered sample (10 g) was added into a glass Petri dish (diameter, 5 cm) according to Morales and van Boekel [24]. The sample was illuminated with D65 artificial daylight (standard angle, 10°) under conditions provided by the manufacturer. Each colour value reported was the average of three determinations. The colour difference between the processed and unprocessed sample (ΔE index) was calculated from the equation:

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (1)$$

where ΔL^* being the brightness difference, Δa^* the redness difference and Δb^* the yellowness difference.

Browning measurement

Browning indices of the sample were determined after an appropriate dilution based on their absorbances at 360 nm and 420 nm measured in a UV-1603 spectrophotometer (Shimadzu, Duisburg, Germany) using a 1 cm optical path cell. Absorbances were corrected for turbidity by subtracting the absorbance at 550 nm. The browning index was defined as the absorbance difference measured at 420 nm and 550 nm and 360 nm and 550 nm, respectively. The results were expressed as absorbance difference per g of sample after the correction for dilution.

Fluorescence measurement

Free fluorescent intermediate compounds (FIC-F) of sample extracts in 0.1 % formic acid (from HMF analysis) were measured at the ex-

citation wavelength of 347 nm and the emission wavelength of 415 nm. The procedure described by MORALES and JIMÉNEZ-PÉREZ [25] was used with some minor modifications. The results were expressed as fluorescence intensity (FI units) per gram of sample after the correction for dilution. A fluorescence detector RF-10AXL (Shimadzu, Kyoto, Japan) was used.

Acrylamide determination by liquid chromatography-mass spectrometry (LC-MS)

For acrylamide extraction from samples of fried rosquillas, the extraction to acetic acid (0.2 mM) and further pre-extraction to ethylacetate to avoid the negative impact of salts in chromatography system were used according to GÖKMEN and ŞENYUVA [26]. A finely ground or homogenized sample (1.000 g) was weighed into a 10 ml centrifuge tube with a cap, and 50 μl of the internal standard and 9 ml of acetic acid extraction solution were added. After shaking by a vortex mixer for 30 s, the mixture was sonicated for 5 min. Then, 500 μl of Carrez solution I (15 g of $(K_4[Fe(CN)_6].3H_2O$ in 100 ml of water) and 500 μl of Carrez solution II (30 g of $(ZnSO_4 .7 H_2O)$ in 100 ml of water) was added and mixed for 1 min. After that, the mixture was centrifuged at 8720 *g* for 10 min. A volume of 5 ml of the clear supernatant was transferred to a separator funnel; 5 ml ethylacetate was added and mixed well. The ethylacetate layer was removed and the extraction step was repeated twice with 5 ml of ethylacetate. Ethylacetate layers were collected and evaporated in a vacuum rotary evaporator at 35 °C to dryness. The residue was dissolved in 1 ml of acetic acid solution and filtered through a 0.45 μm pore size nylon syringe filter.

Liquid chromatography-mass spectrometry (LC-MS) analysis was performed with a HPLC system 1200 series (Agilent Technologies, Santa Clara, California, USA) coupled to an Agilent 6410 Triple Quad detector equipped with electrospray ionisation (ESI) interface. The analytical separation was performed on a Purospher STAR RP-8ec column (150 mm × 4.6 mm, 3 μm particle size) (Merck, Darmstadt, Germany) using an isocratic mixture of 100 ml of acetonitrile and 900 ml of aqueous solution of PFOA (0.05 mM) at a flow rate of 0.5 ml·min^{-1} at ambient temperature. All parameters of the electrospray ionization tandem mass spectrometry (ESI-MS-MS) system were based on in-source generation of the protonated molecular ions of acrylamide and the internal standard (*d3*-acrylamide), as well as collision-induced production of specific fragment ions for multiple reaction monitoring (MRM) experiments (transition for acrylamide: 72 → 55, transition for *d3*-acrylamide: 75 → 58) and the following instrumental parameters were used for acrylamide analysis in the ESI+ mode: drying gas (N_2) flow of 8 l·min^{-1}, gas temperature of 350 °C, nebulizer pressure of 345 kPa, capillary voltage of 2.5 kV, fragmentor of 80 V, collision energy of 5 eV, dwell 50 ms. Calibration was performed by diluting the acrylamide stock solution (0.02 g in 100 ml of methanol) in the range of 50–2000 ng·10 ml^{-1} with 50 μl of the internal standard (*d3*-acrylamide).

Amino acids determination by LC-MS

For the determination of amino acids, the method according to ÖZCAN and ŞENYUVA [27] was used. A finely ground sample of rosquillas (1.000 g) was transferred into a 10 ml centrifuge tube with a cap. A volume of 10 ml of 0.2 mM acetic acid was added to the samples, shaken by a vortex mixer for 2 min and centrifuged at –4 °C at 1910 *g* for 10 min. One ml of the clear supernatant was transferred to a 10 ml volumetric flask, 50 μl of internal standard stock solution (*d3*-glutamic acid) was added and filled up with 0.2 mM acetic acid. It was filtered through 0.45 μm pore size nylon syringe filter prior to LC-MS analysis. Internal standard (50 μl) was added directly to the sample and the same procedure was followed.

The LC/ESI-MS-MS analyses for quantification of four free amino acids were performed by HPLC system 1200 series (Agilent Technologies) coupled to an Agilent 6410 Triple Quad detector equipped with ESI interface. The analytical separation was performed on a Purospher STAR RP-8ec (150 mm × 4.6 mm, 3 μm particle size) using an isocratic mixture of 100 ml of acetonitrile and 900 ml of aqueous solution of PFOA (0.05 mM) at a flow rate 0.5 ml·min^{-1} at the ambient temperature. All parameters of the ESI-MS-MS system were based on in-source generation of the protonated molecular ions of the four amino acids measured and the internal standard (*d3*-glutamic acid), as well as collision-induced production of amino acid-specific fragment ions for MRM experiments. The following instrumental parameters were used for LC-MS-MS analysis of amino acid in the positive MRM mode: drying gas (N_2), flow of 8 l·min^{-1}, gas temperature of 320 °C, nebulizer pressure of 345 kPa, capillary voltage of 3 kV, fragmentor of 40, collision energy of 12 eV, dwell 50 ms.

Saccharides determination by HPLC with refractive index detection (RI)

Contents of glucose, fructose and saccharose were determined using HPLC with a refractive index (RI) detector PU 4003 (Pye Unicam, Cam-

bridge, United Kingdom) on a Polymer IEX Ca^{2+} form column (250 mm × 8 mm, 8 μm particle size; Watrex, Berlin, Germany) at 90 °C. Deionized water was used as a mobile phase with a flow rate of 0.5 ml·min^{-1}. Sample volumes of 20 μl were injected. Each sample of 1.000 g was twice extracted with 10 ml of deionized water. Sorbitol was used as an internal standard. The mixture was mixed by a vortex mixer for 2 min and centrifuged at 1 910 *g* for 10 min. The extract was filtered through a 0.45 μm pore size nylon syringe filter.

HMF measurement

HMF content in samples was measured according to Rufian-Henares et al. [28]. The ground sample (500 mg) was suspended in 5 ml of 0.1 % formic acid in a 10 ml centrifuge tube. The tube was shaken for 10 s by a vortex mixer and clarified with 0.25 ml of potassium ferrocyanide (15%, w/v) and zinc acetate (30%, w/v) solutions. The resulting mixture was centrifuged at 2 000 *g* for 10 min at 4 °C. The supernatant was collected in a 10 ml volumetric flask and two further extractions were performed using 2 ml of 0.1% formic acid. The supernatants were mixed and centrifuged again. Analysis for HMF was made by HPLC in the filtered (0.45 μm) solution.

The HPLC system consisted of a MD-420 pump, a MD-465 autosampler, a MD-432 ultraviolet-visible detector and DT-450/MT v. 3.90 computing integrator connected to a PC, all from Kronton Instruments (Milan, Italy). A mixture of acetonitrile in 0.1% formic acid (5%, v/v) delivered at a flow rate of 1.0 ml·min^{-1} under isocratic conditions through the analytical column (Extrasyl-ODS2, 250 mm × 4 mm, 5 μm particle size; Tecknokroma, Barcelona, Spain) at 32 °C was used as a mobile phase. The UV detector was set at 280 nm and 20 μl of the extract was injected. HMF was quantified using the external standard method within the range 1–50 μM and 30–120 μM, respectively.

Furosine measurement

Content of furosine in samples was measured according to Delgado et al. [29] with some modifications. An amount of 30 mg of the sample was hydrolysed with 4 ml of 7.95 N HCl at 110 °C for 23 h in a Pyrex screw-cap vial with a polytetrafluoroethylene (PTFE) septum. Hydrolysis tubes were sealed under nitrogen. The hydrolysates were cooled at room temperature and centrifuged at 14 000 *g* for 10 min at 4 °C. A 0.5 ml portion of the supernatant was applied to a Sep-Pak C_{18} cartridge (Waters, Milford, Maryland, USA), prewetted with 2.5 ml of methanol and 5 ml of deionized water, and then it was eluted with 3 ml of 3 M HCl. The sample was dried in a vacuum rotary evaporator at 65–70 °C and, subsequently, dissolved in 1 ml of 0.2% formic acid. Analyses for furosine were made by HPLC in the filtered (0.45 μm) solution. The HPLC system consisted of a MD-420 pump, a MD-465 autosampler, a MD-432 ultraviolet-visible detector and DT-450/MT v. 3.90 computing integrator connected to a PC, all from Kronton Instruments. An ODS2-Mediterranea-Sea analytical column (250 mm × 4 mm, 5 μm particle size; Tecknokroma, Barcelona, Spain) was used at 35 °C, isocratically eluted at a 1.0 ml·min^{-1} flow rate with a degassed mobile phase prepared with 5 mM sodium heptane sulphonate containing 20% of acetonitrile and 0.2% of formic acid. The injection volume was 20 μl and the detection was performed at 280 nm. Furosine was quantified by the external standard method. A calibration curve was constructed using a stock solution (1.0 mg·ml^{-1} of furosine) in the range 1–25 mg·l^{-1}.

Free radical-scavenging capacity determination by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay

For the determination of the free radical-scavenging capacity expressed as Trolox equivalent antioxidant capacity (TEAC), 1.000 g of a powdered fried sample was transferred into a 10 ml glass tube and the reaction was started by adding 9 ml of 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) reagent solution with a concentration of 0.1 mM. The solution was mixed by hand for 3 min and then was shaken for 45 min using a laboratory shaker Innova 2000 (New Brunswick Scientific, Edison, New Jersey, USA) at 3.3 Hz and subsequently centrifuged at 500 *g* for 10 min. The absorbance of the clear supernatant was measured at 517 nm according to Serpen et al. [30]. All measurements were performed at exactly 60 min after mixing the sample with the DPPH reagent and expressed as TEAC [mg Trolox per g of sample]. For the spectrophotometric measurement of DPPH radical-scavenging capacity, an UV-VIS spectrophotometer Specord M40 (Carl Zeiss, Jena, Germany) was used at the following conditions: spectral bandwidth 20 cm^{-1}, integration time 1 s, gain 3. For all measurements, a square cell with an optical path length of 1 cm was used.

Statistics

For the statistical purposes, ANOVA – Analysis of Variance (one factor and correlation analyses) was used at a significance level of 0.05. At all determinations, at least three replicates of the sample were analysed.

RESULTS AND DISCUSSION

Studies on pathways of acrylamide generation proved asparagine and reducing saccharides to be the crucial factors determining the following acrylamide formation [8–12]. Moreover, a partial contribution of glutamine and aspartate in this process was assumed [31]. Considering this knowledge, samples of rosquillas were prepared from wheat flour and saccharides by frying in sunflower oil according to three alternatives of the simplified recipe. Ingredients used for sample making are listed in Tab. 1. The modifications in recipes were based on various saccharide contents and composition, when saccharose was substituted for the mixture of glucose and fructose, and in the presence or absence of sodium hydrogen carbonate as a raising agent. The frying process of rosquillas was carried out at two alternative temperatures (180 °C or 200 °C) for three frying times to get an unfinished sample at 4 min, an accurately prepared sample at 6 min, and an excessively fried one at 8 min.

During this process, a decrease in the contents of saccharides and amino acids was observed. On the other hand, the contents of acrylamide and HMF as well as colour parameters and the antioxidant capacity increased. Heat treatment of the samples was associated with a loss of moisture, thus the final water content was recorded in the ranges of 3.2–5.1% at 180 °C, and 2.3–2.8% at 200 °C.

Although acrylamide formation is associated directly with asparagine occurence, it is interesting that a decrease was observed in the contents of all the followed amino acids (Asn, Asp, Gln, Glu), which is evident from amino acid representation in rosquillas prepared from saccharose according to recipe 1 (Fig. 1). Similar behaviour was observed at all recipes and heating conditions. This fact is probably related to amino acid contribution to formation of not only acrylamide but also of other compounds, as well as transamination reactions between Glu and Asp and transamidation among jointed amino acids: Asp and Gln on one side and Asn with Glu on the other side [32].

Concerning acrylamide occurrence, its formation and ways of its minimization in cereal products were subjected to investigation by many authors and reviewed recently by CLAUS et al. [33] and KONINGS et al. [34]. The presented data on the contents of acrylamide in rosquillas confirmed that the formation of acrylamide is evidently time- and temperature-dependent (Fig. 2). The prolongation of frying time from 4 min to 8 min resulted in the increase of acrylamide content from (43 ± 8) $\mu g \cdot kg^{-1}$ to (159 ± 12) $\mu g \cdot kg^{-1}$ at 180 °C, and from (94 ± 3) $\mu g \cdot kg^{-1}$ to (366 ± 5) $\mu g \cdot kg^{-1}$ at 200 °C. This observation point at the fact that the time-temperature control represents the key factor of acrylamide elimination.

As is known from previously published data, inorganic salts such as sodium chloride or sodium bicarbonate display a protective action against acrylamide formation [35, 36]. LEVINE and SMITH [35] reported a 70% reduction of acrylamide in a biscuit cracker model by the substitution of ammonium bicarbonate for sodium bicarbonate. KOLEK et al. [36] described the acrylamide polymerization initiated by sodium chloride addition, which was confirmed by infrared spectrometry.

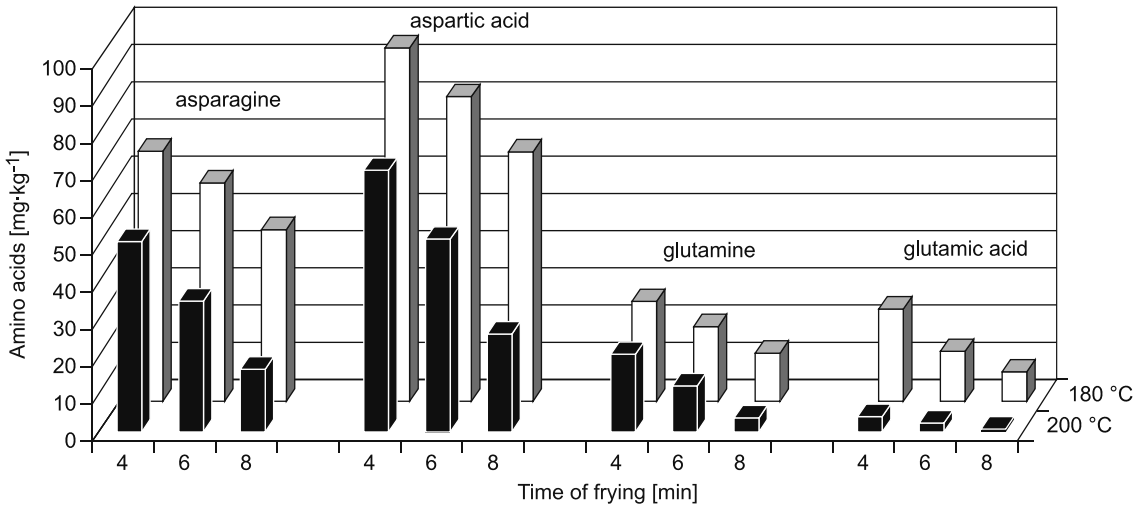

Fig. 1. Decrease in amino acids in rosquillas prepared according to Recipe 1 (dough contained saccharose) fried at 180 °C and 200 °C, respectively, in sunflower oil for 4, 6 and 8 min.

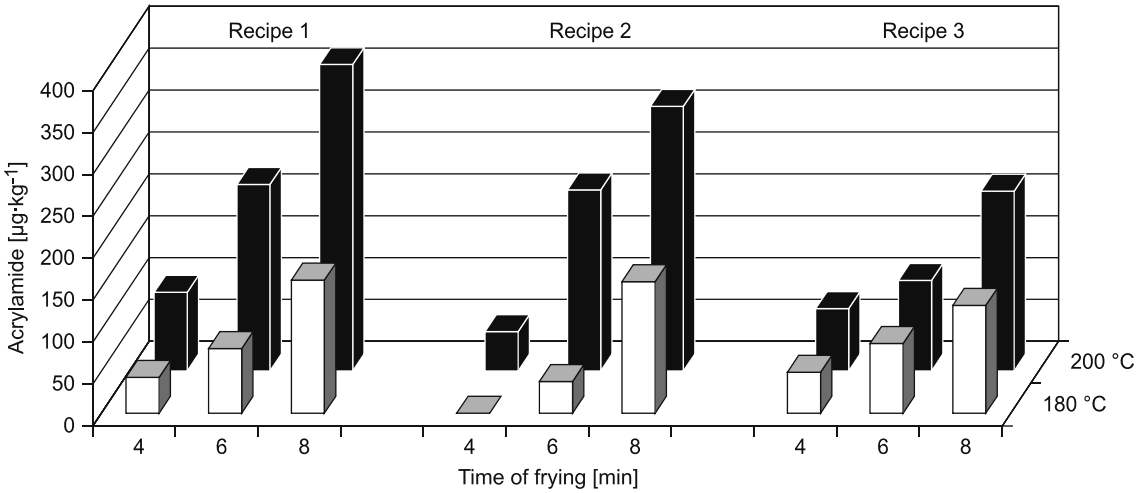

Fig. 2. Formation of acrylamide in rosquillas prepared according to three variations of the recipe, fried at 180 °C and 200 °C, respectively, in sunflower oil for 4, 6 and 8 min.
Recipe 1 – dough contained saccharose, Recipe 2 – dough contained saccharose and $NaHCO_3$, Recipe 3 – dough contained fructose and glucose.

Based on this knowledge, in the presented study sodium hydrogen carbonate was applied into the dough containing saccharose (recipe 2), which resulted in a weak, 13% decrease in acrylamide compared to the recipe without a raising agent addition (recipe 1; Fig. 2). The action of sodium hydrogen carbonate could be explained by lowering of asparagine content in the dough (data not shown) and consequently in a limited acrylamide formation.

Although Asn content is considered to be a crucial factor of acrylamide formation in bakery products, saccharides also play an important role in this process [13, 33]. It was demonstrated by VASS et al. [37] that replacing invert sugar syrup with saccharose in wheat crackers reduced acrylamide by 60%. Similar effects were also observed for gingerbreads [38]. These results were explained by a lack of reactive carbonyls, namely fructose and glucose. On the contrary, TAEYMANS et al. [39] reported that the use of saccharose lead to acrylamide amounts comparable to those obtained with fructose or glucose, due to its thermal decomposition and release of the monomers. The former opinion is supported by our presented results, since in case of saccharose substitution for the mixture of fructose and glucose (recipe 3), formation of only 58% of acrylamide was detected during frying (Fig. 2) in comparison with rosquillas prepared from the dough containing saccharose (recipe 1).

Concerning saccharose importance, AMREIN et al. [38] did not observe any increase in acrylamide content at NH_4HCO_3 addition when saccharose was used instead of invert saccharide syrup, even though it was demonstrated by many authors that NH_4HCO_3 significantly enhanced acrylamide generation in gingerbread, biscuits and crackers [37, 39, 40].

Among other undesirable contaminants resulting from Maillard reaction, HMF was observed. The replacement of saccharose by the mixture of glucose and fructose led to a high level of HMF formation at both temperatures, but was more pronounced at 200 °C (Fig. 3). This observation is in good agreement with the presumption that the formation of HMF is strongly related to the presence of fructose [20].

Furosine (2-furosylmethyl-lysine) is a well-known indicator used to express the extent of damage in processed or stored foods with a long shelf life [41]. Since furosine is considered to be an indicator of the early stage MRP characterized by typical kinetic behaviour with a rapid increase to an apparent maximum followed by an exponential decrease during baking [42], the content of furosine in rosquillas was determined only in samples heated at 180 °C, where the advanced Maillard reaction was not supposed to be completed (Fig. 4). The increase in furosine indicated continuation of early stages of Maillard reaction in the presence of saccharose (recipe 1) and, on the other hand, the decrease in furosine in the the presence of glucose and fructose (recipe 3) indicated the faster development of Maillard reaction and termination of its early stage (Fig. 4).

The colour, taste and odour desired by both producers and consumers of cereal products, together with other positive effects, such as antioxi-

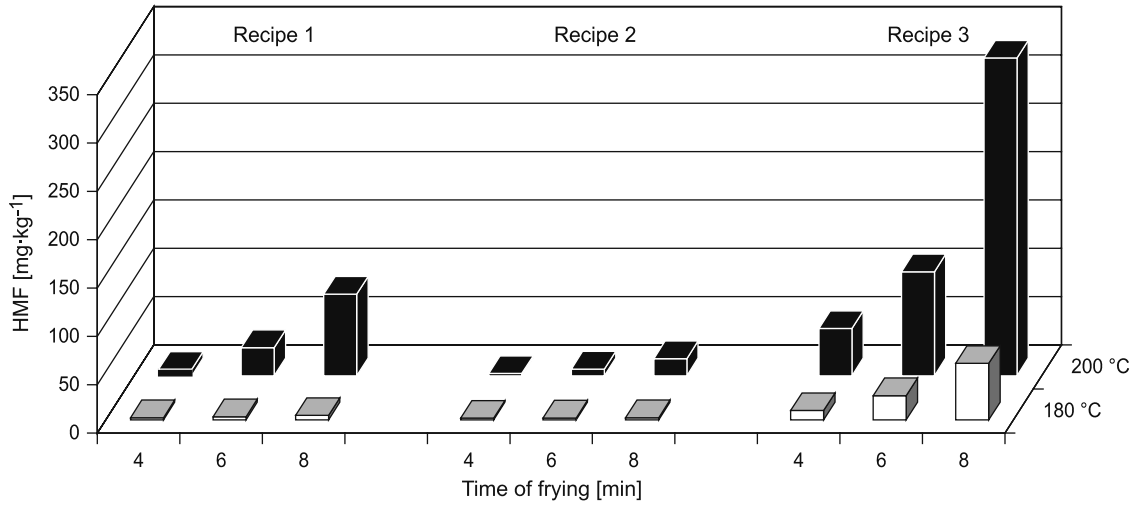

Fig. 3. Formation of hydroxymethylfurfural in rosquillas prepared according to three variations of the recipe, fried at 180 °C and 200 °C, respectively, in sunflower oil for 4, 6 and 8 min.
Recipe 1 – dough contained saccharose, Recipe 2 – dough contained saccharose and $NaHCO_3$, Recipe 3 – dough contained fructose and glucose.

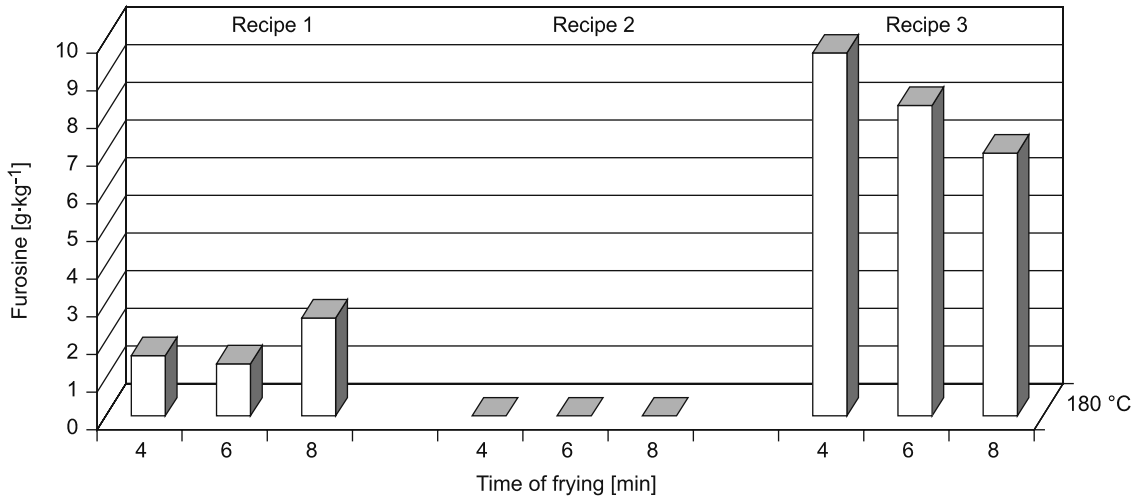

Fig. 4. Formation of furosine in rosquillas prepared according to three variations of therecipe, fried at 180 °C in sunflower oil for 4, 6 and 8 min.
Recipe 1 – dough contained saccharose, Recipe 2 – dough contained saccharose and $NaHCO_3$, Recipe 3 – dough contained fructose and glucose.

dant activity, have also been attributed to MRP. Several studies were focused on the high antioxidant capacity of MRP in model systems and foods such as beer, coffee, potato and bakery products [41, 43, 44]. In these studies it was shown that MRP may contribute greatly to the shelf life of heat-treated foods. In vitro studies demonstrated that MRP may offer a substantial health-promoting activity as they can act as reducing agents, metal chelators and radical scavengers. Nevertheless, the development of colour is an important and obvious consequence of the Maillard reaction, while browning is not directly related to the free radical-scavenging properties of MRP formed at prolonged heating. However, previously published data showed that fluorescence was more effective than browning measurement to follow the formation of MRP with free radical-scavenging activities [25]. In the presented study, the colour formation expressed as ΔE values (trichromatic coordinates from CIELab system), browning values determined as the absorbance at 420 nm and 360 nm, and free fluorescence intermediate compounds (FIC-F) as precursors of brown pigments melanoidins formed

Tab. 2. Correlation between free radical-scavenging capacities, acrylamide contents and colour parameters in rosquillas after frying at 180 °C and 200 °C, respectively.

	TEAC / A_{420}	TEAC / A_{360}	TEAC / ΔE	TEAC / FIC-F	TEAC / acrylamide
Recipe 1	0.832604	0.967548	0.778943	0.981343	0.895704
Recipe 2	0.668541	0.896102	0.866643	0.765922	0.762377
Recipe 3	0.548964	0.554968	–0.56916	0.32881	0.238622
	acrylamide / A_{420}	acrylamide / A_{360}	acrylamide / ΔE	acrylamide / FIC-F	acrylamide / TEAC
Recipe 1	0.968076	0.925118	0.915933	0.900207	0.895704
Recipe 2	0.915981	0.920762	0.94864	0.912766	0.762377
Recipe 3	0.886755	0.882526	–0.90921	0.979548	0.238622

TEAC – Trolox equivalent of the antioxidant capacity in mg of Trolox per g of sample, A_{420} and A_{360} – browning expressed as absorbance at 420 nm and 360 nm per gram of sample, ΔE – total colour difference, FIC-F - free fluorescent compounds in fluorescence intensity units per g of sample, acrylamide in $\mu g.kg^{-1}$.
Recipe 1 – dough contained saccharose, Recipe 2 – dough contained saccharose and $NaHCO_3$, Recipe 3 – dough contained glucose and fructose.

during Maillard reaction, were compared with an antioxidant activity measured as a DPPH free radical-scavenging capacity. It was found that in case of rosquillas containing saccharose (recipes 1 and 2), brown colour development was correlated well with the radical-scavenging capacity (Tab. 2) using all determined colour parameters (ΔE values, browning values, fluorescence values), with high correlation coefficients (between 0.807 and 0.981). In case of the mixture of glucose and fructose, rapid development of brown colour was not accompanied with an appropriate occurrence of free radical-scavenging capacity, which was reflected by a substantial decrease in the values of the correlation coefficient (Tab. 2). Moreover, colour parameters showed a good correlation with the final acrylamide content, which was evident from high correlation coefficients (between 0.882 and 0.979) for all recipes (Tab. 2). These findings pointed at the importance of colour determination as a pertinent tool of acrylamide control.

CONCLUSION

Although heat treatment of foods is related to formation of possibly harmful compounds (acrylamide, 5-(hydroxymethyl)-2-furfural), on the other hand, desirable properties (colour, browning, radical-scavenging capacity) develop during Maillard reaction. Since precursors of acrylamide and HMF (amino acids, saccharides) are present in the dough, modifications in dough formulation and time-temperature control during the baking process may be used to decrease the content of these compounds in fried fine bakery products. If acrylamide and HMF accumulation in fried products is attempted to be decreased, attention should be paid to the following points:

1. prolongation of heat treatment and overheating of foods result in higher contents of acrylamide and HMF,
2. presence of saccharose as a non-reducing saccharide does not seem to be a preventing factor from high acrylamide formation,
3. presence of fructose is related to high HMF formation,
4. colour development is in a good correlation with a positive free radical-scavenging capacity in the presence of saccharose, but not glucose and fructose.

Among other possibilities of avoiding acrylamide formation, asparagine elimination by the enzyme L-asparaginase should be mentioned. This process has no undesirable impact on sensory characteristics of final products and was successfully applied in various food products including fried bakery products, rosquillas [45].

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