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Effect of Thermal Processing on Simultaneous Formation of Acrylamide and Hydroxymethylfurfural in Plum Purée

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The formation of acrylamide (ACR) and hydroxymethylfurfural (HMF) at different time and temperature combination in plum purée derived from two species was investigated. An optimized method for reducing ACR and HMF formation in thermally-treated plum purée was developed using a Central Composite Design model. Precursors of contaminants and their influence on the heating of plum purée were evaluated as well. The contaminants content was determined in thirteen running variants in the temperature range of 59.3–200.7°C, and heating time between 5.9 and 34.1 min. The model allowed establishing that the lowest ACR content was reached at 5.9-min exposure time and 130°C temperature, for both plum species (3.91 μg/kg and 8.73 μg/kg for *Prunus cerasifera* (P1) and *Prunus domestica* (P2), respectively). The lowest quantity of HMF was found at 20-min exposure time and 59.3°C temperature for both plum species (0.25 mg/kg and 0.18 mg/kg for P1 and P2, respectively). The results obtained allowed predicting the ACR/HMF levels in plum purée at different heating conditions.

INTRODUCTION

Plums are fruits rich in vitamins, minerals, antioxidants, and other bioactive components, with numerous health benefits [Birwal *et al.,* 2017]. Consumption of fresh thermally-treated plums (prunes, juice, compote, and jam) may prevent from anemia, constipation, obesity, and cardiovascular diseases [Sahamishirazi *et al.,* 2017]. During thermal treatment of plums, beside the desired sensorial properties, different compounds can be found such as advanced glycation end products (AGEs) and low-molecular-mass browning products such as acrylamide (ACR) and 5-hydroxymethylfurfural (HMF) [Nguyen *et al.,* 2016]. Their possible mutagenic, carcinogenic, and/or cytotoxic effects have been proved in previous research [Nursten, 2005]. In 2002, the Swedish National Food Administration added ACR to the list of food-borne toxic compounds, which have been found in high amounts in some heat-treated, carbohydrate-rich foods such as potato chips and crisps, coffee and bread [Swedish National Food Administration, 2002], and later in hazelnuts and almonds [Amrein *et al.,* 2007], dried fruits [Kukurová *et al.,* 2015], and vegetables [Constantin *et al.,* 2014]. Different pathways for ACR formation in foods have been reported such as Maillard reaction between free asparagine as the primary precursor and sugars [Zyzak *et al.,* 2003; Blank *et al.,* 2005]; formation from acrolein and acrylic acid [Yasuhara *et al.,* 2003]; formation from wheat gluten [Claus *et al.,* 2006], and from 3-aminopropionamide (3-APA) [Granvogl & Schieberle, 2006].

HMF is an organic compound included into the class of furans. It is formed as an intermediate in the Maillard reaction or through fructose dehydration under acidic conditions at elevated temperatures. HMF was found in bakery products, honey, malt, fruit products, coffee, vinegar, and dried fruit [Capuano & Fogliano, 2011]. In literature, a high variability in ACR and HMF contents has been reported between different studied products, which was mainly influenced by the difference in food composition including precursors' content (free asparagine and reducing sugars) present in raw materials and in process conditions applied (pH, water activity, temperature/time combination, presence of divalent cations) [Gökmen *et al.,* 2008]. These two neo-formed contaminants (NFC) are very interesting because of their high occurrence in food and toxicological potential such as mutagenic, carcinogenic and cytotoxic effects [Capuano & Fogliano, 2011]. The purpose of this study was to find out information on acrylamide and 5-hydroxymethylfurfural formation during

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thermal treatment of plum purée at different combinations of time and temperature. This study will help food processors to establish the optimal temperature/time combination to obtain lower amounts of ACR and HMF in the final product.

MATERIALS AND METHODS

Reagents and chemicals

Internal standards: 2,2,3-*d3*-2-propenamide (*d3*-ACR) and 2,4,4-*d3*-glutamic acid (*d3*-Glu), purity 97–98% were achieved from Cambridge Isotope Laboratories (Andover, Maryland, USA); standard of acrylamide (ACR), purity 99%, 21 L-Amino Acids Kit: L-alanine ≥98% (Ala), L-arginine monohydrochloride ≥98% (Arg), L-asparagine ≥98% (Asn), L-aspartic acid ≥98% (Asp), L-cysteine hydrochloride anhydrous ≥98% (Cys), L-glutamine ≥99% (Gln), L-glutamic acid ≥99% (Glu), L-glycine ≥99% (Gly), L-histidine monohydrochloride monohydrate ≥98% (His), *trans*-4-hydroxy-L--proline ≥98% (Hyp), L-isoleucine ≥98% (Ile), L-leucine ≥98% (Leu), L-lysine monohydrochloride ≥98% (Lys), L-methionine ≥98% (Met), L-phenylalanine ≥98% (Phe), L-proline ≥99% (Pro), L-serine ≥99% (Ser), L-threonine ≥98% (Thr), L-tryptophan ≥98% (Trp), L-tyrosine ≥98% (Tyr), L-valine ≥98% (Val), and L-ornithine ≥98% (Orn), hydroxymethylfurfural (HMF) 99% purity, perfluorooctanoic acid (PFOA) 96%, and acetonitrile HPLC gradient grade were purchased from Sigma-Aldrich (Steinheim, Germany); ethyl acetate and acetic acid glacial grade were purchased from Fischer Scientific (Loughborough, UK); methanol HPLC-grade, potassium hexacyanoferrate trihydrate, and zinc sulfate heptahydrate were achieved from Merck (Schuchardt, Germany). Nylon syringe filters (0.45 μm) were obtained from Waters (Milford, Milford, MA, USA).

Plant material

Fruits of two plum species: *Prunus cerasifera* – cherry plum (P1) and *Prunus domestica* Angeleno (P2), were used in this study. The selected plums represented the offseason species available on the Romanian market. Plums have been purchased on the local market and stored at 4°C before analysis. The dry matter content was determined with a classic thermogravimetric method (removing the water using an oven at 105°C temperature) and revealed the following values in plums: 10.18% for P1 and 9.03% for P2. Plums were washed, homogenized at 10,000 rpm for 15 s (Grindomix Retsch GM200), and heat treated according to the experimental model.

Extraction and analysis of plum purée amino acids

For the determination of amino acids, 2 g of plum purée were weighed into a 10-mL centrifuge tube. The extraction solution (20 mL, 0.1 % acetic acid (v/v)) was added to the sample and stirred for 30 min at 150 rpm (Heidolph Unimax 2010, Schwabach, Germany) and centrifuged at 19,621×*g* for 10 min (Sigma 2–16 KC, Germany). The samples were diluted using the following protocol: 100 μL of the clear supernatant of each sample were transferred to a 10-mL volumetric flask, 50 μL of *d3*-Glu (stock solution 10-fold diluted) were added, and the flask was filled up with 0.1% acetic acid (v/v). The extract was filtered through a 0.45 μm nylon syringe filter before the LC/MS analysis. The LC/ESI-MS-MS analyses for quantification of free amino acids profile were performed in the HPLC system 1200 series coupled to an Agilent 6410 Triple Quad detector equipped with ESI interface (Agilent Technologies, Santa Clara, California, USA). The analytical separation was performed on a Purospher STAR RP-8ec column (4.6×150 mm, 3μm particle size) (Merck, Darmstadt, Germany) using an isocratic elution with a 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 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 the amino acids measured and the internal standard (*d3*-Glu) as well as collision-induced production of amino acid-specific fragment ions for Multiple Reaction Monitoring (MRM) experiments [Constantin *et al.,* 2014].

Reducing sugars content determination

Determination of the content of reducing sugars was performed using the 3,5-dinitrosalicylic acid (DNS) according to AOAC method [1995]. Briefly, 2 mL of the sample homogenized with 2 mL of 0.04 M DNS solution were maintained in a boiling-water bath for 10 min. After cooling, the contents of the tubes were brought to the volume of 10 mL with distilled water. The absorbance of the mixture was measured at 535 nm wavelength against a prepared blank using a Jenway 6506 UV-Vis Spectrophotometer (Cole-Parmer, Stone, United Kingdom). The results were expressed as mg glucose/g DW.

Thermal treatment and experimental design

Plum purée was subjected to heat treatment using a thermostat (Liebisch Labortechnik, Germany), in a range of temperatures between 59.3 and 200.7°C, and heating times between 5.9 and 34.1 min according to the experimental model parameters of which are presented in Table 1. Central Composite Design (CCD) and response surface modeling have been used to optimize the thermal treatment of plum purée to obtain ACR and HMF. CCD builds a quadratic model for response variables. The design involves three distinct sets of steps: a factorial design on the variables studied, a set of focal points and a set of points or samples axial stay. The design investigates five levels of each variable studied. Circumscribed data set was used, with a distance of ±1.4142 proven stars. The experiments were carried out in the order given by the software to determine the influence of external factors in the analysis. Two parameters were analyzed such as temperature and time of thermal treatment, and contents of ACR and HMF were selected as the answers Design-Expert® software (Stat-Ease, Inc.) was used for data analysis.

The experimental conditions can be described by equation (1).

$$R = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2 \quad (1)$$

where: A, B are independent variables studied and b_0 – intercept, b_1-b_5 represent regression coefficients, constants for

TABLE 1. Matrix of experimental design (coded levels and real values) with responses in terms of acrylamide (ACR) and hydroxymethylfurfural (HMF) content in purée made of *Prunus cerasifera* (P1) and *Prunus domestica* (P2) plums.

Run	Coded levels		Actual levels		Plum purée P1		Plum purée P2	
	Temperature (°C) (A)	Time (min) (B)	Temperature (°C) (A)	Time (min) (B)	ACR (μg/kg DW)	HMF (mg/kg DW)	ACR (μg/kg DW)	HMF (mg/kg DW)
1	0	0	130	20	21.50	34.73	9.92	4.68
2	-1	+1	80	30	35.28	25.38	50.55	2.85
3	-1.41	0	59.3	20	8.49	0.25	31.72	0.18
4	-1	-1	80	10	5.48	0.34	15.99	0.51
5	0	0	130	20	30.18	34.69	12.30	4.53
6	0	0	130	20	27.37	34.53	9.85	5.48
7	0	0	130	20	22.74	32.75	11.57	3.94
8	0	-1.41	130	5.9	3.91	35.17	8.73	0.45
9	+1.41	0	200.7	20	1024.89	139.06	634.75	90.40
10	0	0	130	20	22.70	31.86	15.07	3.79
11	+1	0	180	30	876.68	100.72	596.89	92.88
12	0	+1.41	130	34.1	552.29	95.36	79.42	25.19
13	+1	-1	180	10	447.77	116.89	263.18	57.03

the effect of the general process, the linear and quadratic effects of each independent variable, as well as the interaction effects of the variables on the content of ACR and HMF.

ACR content determination

After heat processing of plum purée, ACR was extracted with 30 mL of 0.1% acetic acid (v/v) and further pre-extracted with ethyl acetate to avoid the negative impact of salts in the chromatographic system according to procedures published before by Constantin *et al.* [2014] and Ciesarová *et al.* [2009]. Acetic acid extraction step was repeated three times. The samples were shaken for 1 min and then clarified with 1 mL of Carrez solution I (15% potassium ferrocyanide) and 1 mL of Carrez solution II (30% zinc acetate). The acetic acid extracts were collected and brought to a total volume of 100 mL with 0.1% acetic acid (v/v). After mixing for 1 min and sonication for 10 min, the samples were shaken by a vortex mixer for 1 min, sonicated for 5 min, and centrifuged at -5°C for 10 min at 19,621×*g* (Sigma 2–16 KC, Germany). A volume of 5 mL of the clear supernatant was transferred to a test tube with the addition of 100 μL of internal standard *d3*-ACR solution (2 mg in 100 mL of water) and 5 mL of ethyl acetate, and mixed well for 1 min. The ethyl acetate top layer was removed to a clean test tube. The step of pre-extraction with ethyl acetate was repeated three times, and all the ethyl acetate layers were collected and evaporated under vacuum at 35°C to dryness. The dry residue was dissolved in 1 mL of 0.1% acetic acid (v/v) and filtered through a nylon syringe filter with 0.45 μm pore size to glass vials before LC--MS analysis.

The LC/ESI-MS-MS technique using the 1260 Infinity HPLC system coupled to 6410 Triple Quad LC/MS equipped with ESI interface (Agilent Technologies, Santa Clara, California, USA) was applied. The analytical separation was performed on Atlantis dC18 column (30×100 mm, 3 μm pore size, Waters, Milford, MA, USA) using isocratic elution of 1% methanol (v/v) and 0.2% acetic acid (v/v) in water as a mobile phase at 0.4 mL/min flow rate at 25°C. 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 ACR and the internal standard (*d3*-ACR), as well as collision-induced production of specific fragment ions for MRM experiments (transition for ACR: 72 → 55, transition for *d3*-ACR: 75 → 58). The following instrumental parameters were used for ACR analysis in the ESI+ mode: drying gas (N_2) flow of 8 L/min, 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, and dwell 50 ms. Calibration was performed by diluting the ACR stock solution (5 mg in 100 mL of water) in the range of 10 – 2000 ng/10 mL with 50 μL of the internal standard (*d3*-ACR).

HMF content determination

After heat processing of plum purée, HMF was extracted with 30 mL of a methanol: water mixture (80:20, v/v). The mixture was sonicated for 5 min, centrifuged for 10 min at 19,621×*g* (Sigma 2–16 KC, Germany) and filtered through 0.45 μm nylon membrane syringe filters. Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, California, USA) equipped with UV/VIS detector (DAD) was used for HMF analysis. The chromatographic separation was performed on C18 SB column (4.6×250mm, particle size 5 μm, Waters, Milford, MA, USA) using the gradient elution at a flow

TABLE 2. Contents of amino acids and reducing sugar in purée made of *Prunus cerasifera* (P1) and *Prunus domestica* (P2) plums.

Compound		Plum purée	
		P1	P2
Amino acids (mg/kg DW)	Hyp*	0.28±0.09	0.00±0.00
	Asp*	5.56±0.10	4.93±0.10
	Pro*	51.93±0.75	39.65±0.45
	Asn*	178.41±1.01	138.78±0.35
	Ser	5.95±0.02	5.88±0.19
	Gln*	34.08±0.36	91.51±0.01
	Thr*	3.32±0.05	3.40±0.07
	Glu*	5.64±0.10	8.59±0.23
	Gly*	0.73±0.08	0.72±0.11
	Ala*	10.22±0.22	13.15±0.18
	Val	3.06±0.02	5.05±0.06
	Met	0.00±0.00	0.00±0.00
	Tyr	0.00±0.00	0.00±0.00
	Ile*	2.38±0.02	6.08±0.03
	Leu*	2.40±0.02	6.07±0.03
	Phe*	1.40±0.05	3.58±0.02
	His*	2.71±0.23	1.34±0.05
	Orn*	0.27±0.03	0.38±0.01
	Lys*	0.54±0.00	0.97±0.00
	Arg*	0.32±0.05	0.79±0.08
	Trp	0.16±0.00	0.41±0.00
	Sum	14.75±0.11	15.77±0.00
Reducing sugars (RS) (mg/g DW)	RS*	11.12±1.03	9.18±0.34

Explanations: Hyp – 4-trans-hydroxyproline, Asp – aspartic acid, Pro – proline, Asn – asparagine, Ser –serine, Gln – glutamine, Thr –threonine, Glu – glutamic acid, Gly – glycine, Ala – alanine, Val – valine, Met – methionine, Tyr – tyrosine, Ile – isoleucine, Leu – leucine, Phe – phenylalanine, His – histidine, Orn – ornithine, Lys – lysine, Arg – arginine, and Trp – tryptophan.

* indicates significant difference between samples ($p<0.05$); DW – dry weight.

rate of 0.8 mL/min at 25°C. The mobile phase consisted of % of methanol (A), 0.01 M H_3PO_4 (B), and acetonitrile (C). Gradient composition for HMF determination was applied as following: 0–1.5 min, 0–2% A, 100–95% B, 0–3% C; 1.5–2.1 min, 2% A, 95% B, 3% C; 2.1–3.0 min, 2–8% A, 95–86% B, 3–6% C; 3.0–11.0 min, 8% A, 86% B, 6% C; 11.0––11.5 min, 8–94% A, 86–0% B, 6% C; 11.5–20.0 min, 94% A, 0% B, 6% C; 20.0–20.1 min, 94–2% A, 0–95% B, 6–3% C; 20.1–30.0 min, 2% A, 95% B, 3% C.

The HMF was detected at its absorption maximum of 280 nm and quantified using external calibration curve in the range from 0.05 to 1.0 μg/mL.

Statistical analysis

Data were analyzed using multivariate data analysis and Design Expert v. 10.1 software from Design-Expert® (Stat-Ease, Inc., Minnesota, USA) and by paired t-test using SPSS19.0 (IBM, New York, NY, USA).

RESULTS AND DISCUSSION

Contents of amino acids and reducing sugars in plum purée

The purées from fresh red plum were analyzed for ACR and HMF precursors, and the results of determinations of contents of amino acids and reducing sugars are presented in Table 2. The amino acids analysis showed a high asparagine content in both plums compared to other amino acids (178.41±1.01 mg/kg DW for P1 and 138.78±0.35 mg/kg DW for P2). It is known that asparagine is a crucial participant in the production of ACR in the Maillard reaction [Mottram *et al.,* 2002]. Its high content in P1 and P2 plum purée may be considered responsible for the ACR formation.

Reducing sugars content in P1 and P2 plums purée was 11.12±1.03 mg/g DW and 9.18±0.34 mg/g DW, respectively. Leong & Oey [2012] obtained similar results (9.70±0.98 – 16.54±0.04 mg/g DW) for plum from the Otago region (South Island, New Zealand). The presence of reducing sugars is essential in both ACR and HMF formation, as they form a Schiff base with asparagine, and then by decarboxylation ACR, while HMF is formed by their caramelization or thermal dehydration [Friedman, 1996; Abraham *et al.,* 2011].

Thermal treatment optimization

The Central Composite Design (CCD) and surface response modeling were used to determine the optimal parameters (temperature and time) of the thermal treatment of plum purée, and to achieve minimal contents of ACR and HMF. Table 1 presents the matrix of the complete CCD used in optimization with actual values of the main variables studied, and the corresponding values of the ACR and HMF content measured. The optimized coded model for contaminants was represented using ANOVA (Table 3 and Table 4).

Acrylamide formation

ACR is generally formed in thermally treated foods (>120°C), with a high level of carbohydrates [Tareke *et al.,* 2000, 2002; Gökmen, 2015]. As it can be seen in Table 4, the quantities of ACR in the thermally treated plum purée have considerably fluctuated. These differences could depend on plum species, their chemical composition (reducing sugar and amino acid content), and processing conditions (time and temperature) [Becalski *et al.,* 2011]. The model selected revealed the concentration of the ACR formed in the treated purée to highly depend on the selected parameters. A low ACR content was obtained for the eighth running variant (130 min/5.6°C) for both plum species (Table 1). Furthermore, the content of ACR determined in sample P1 was almost 2-fold higher when compared to the sample P2. This significant difference can be due to the higher content of asparagine and reducing sugars. ACR was suggested to be formed by the specific amino acid route due to the suf-

TABLE 3. ANOVA for the square surface of the acrylamide (ACR) formation during thermal treatment of purée made of *Prunus cerasifera* (P1) and *Prunus domestica* (P2) plums.

Statistical parameters	Sum of Squares	df	Mean Square	F Value	p-value
Plum purée P1					
Model	1.578E+006	5	3.156E+005	101.10	< 0.0001
A-Temperature	*9.255E+005*	*1*	*9.255E+005*	*296.54*	*< 0.0001*
B-Time	*1.904E+005*	*1*	*1.904E+005*	*61.01*	*0.0001*
AB	*39823.27*	*1*	*39823.27*	*12.76*	*0.0091*
A^2	*3.740E+005*	*1*	*3.740E+005*	*119.83*	*< 0.0001*
B^2	*88163.21*	*1*	*88163.21*	*28.25*	*0.0011*
Residual	21847.92	7	3121.13		
Lack of Fit	*21792.95*	*3*	*7264.32*	*528.60*	*< 0.0001*
Pure Error	*54.97*	*4*	*13.74*		
R-Squared			0.9863		
Adj R-Squared			0.9766		
Plum purée P2					
Model	*5.933E+005*	*5*	*1.187E+005*	*63.15*	*< 0.0001*
A-Temperature	*3.388E+005*	*1*	*3.388E+005*	*180.30*	*< 0.0001*
B-Time	*27406.26*	*1*	*27406.26*	*14.58*	*0.0066*
AB	*22371.76*	*1*	*22371.76*	*11.91*	*0.0107*
A^2	*2.046E+005*	*1*	*2.046E+005*	*108.88*	*< 0.0001*
B^2	*5039.41*	*1*	*5039.41*	*2.68*	*0.1455*
Residual	*13153.71*	*7*	*1879.10*		
Lack of Fit	*13135.39*	*3*	*4378.46*	*955.88*	*< 0.0001*
Pure Error	*18.32*	*4*	*4.58*		
R-Squared			0.9783		
Adj R-Squared			0.9628		

ficient contents of reducing sugars related to the content of asparagine. The CCD allowed estimating equations which enable predicting the most suitable models for the production of ACR for both samples (P1 and P2) as follows (Eq. 2 and 3):

$$\text{P1 ACR} = +24.90 + 340.14\,A + 154.28\,B + 99.78\,AB + 231.87\,A^2 + 112.58\,B^2 \quad (2)$$

$$\text{P2 ACR} = +11.74 + 205.79\,A + 58.53\,B + 74.79\,AB + 171.49\,A^2 + 26.91\,B^2 \quad (3)$$

Optimized coding models for ACR contents were represented by regression analysis and variance analysis (ANOVA), and the quadratic models were applied. From the ANOVA (Table 3), it can be seen that both models (P1 and P2) fitted well to optimization data (R^2=0.9863 and R^2 is 0.9783, respectively), and the F values (101.10 and 63.15) indicated that the pattern was significant. In this case, the significant model terms were: A, B, AB, A^2, B^2 for P1 and A, B, AB, A^2 for P2. The ACR content determined in samples P1 and P2 was positively correlated with all individual terms, with the greatest influence of the A^2 (square of temperature response) for P1, and A for P2 sample.

Figure 1 shows the correlative effect of temperature and time on ACR formation. The content of ACR increased as the processing temperature of the plum purée increased. As it can be seen from the response surface graph (Figure 1A and B), ACR formation was minimal at the shortest time of thermal treatment, between 10 and 20 min. For the plum purée P1, the lowest ACR formation was at the minimum exposure time, between 15 and 20 min. Moreover, an increase in ACR formation at high temperatures (≥180°C) was correlated with an extended thermal treatment interval (≥25 min) (Figure 1A). For the plum purée P2, the combined effect of time and temperature revealed an increased effect on ACR formation at temperatures above 180°C and up to 25 min (Fig-

TABLE 4. ANOVA for the square surface of the hydroxymethylfurfural (HMF) formation during thermal treatment of purée made of *Prunus cerasifera* (P1) and *Prunus domestica* (P2) plums.

Statistical parameters	Sum of Squares	df	Mean Square	F Value	p-value
Plum purée P1					
Model	23234.07	5	4646.81	39.46	< 0.0001
A-Temperature	*18836.10*	*1*	*18836.10*	*159.97*	*< 0.0001*
B-Time	*1104.44*	*1*	*1104.44*	*9.38*	*0.0183*
AB	*424.31*	*1*	*424.31*	*3.60*	*0.0995*
A^2	*1851.49*	*1*	*1851.49*	*15.72*	*0.0054*
B^2	*1386.95*	*1*	*1386.95*	*11.78*	*0.0110*
Residual	824.25	7	117.75		
Lack of Fit	*817.24*	*3*	*272.41*	*155.44*	*0.0001*
Pure Error	*7.01*	*4*	*1.75*		
R-Squared			0.9657		
Adj R-Squared			0.9413		
Plum purée P2					
Model	14022.45	5	2804.49	89.37	< 0.0001
A-Temperature	*9394.83*	*1*	*9394.83*	*299.38*	*< 0.0001*
B-Time	*669.31*	*1*	*669.31*	*21.33*	*0.0024*
AB	*280.75*	*1*	*280.75*	*8.95*	*0.0202*
A^2	*3589.86*	*1*	*3589.86*	*114.40*	*< 0.0001*
B^2	*292.43*	*1*	*292.43*	*9.32*	*0.0185*
Residual	219.66	7	31.38		
Lack of Fit	*217.86*	*3*	*72.62*	*161.41*	*0.0001*
Pure Error	*1.80*	*4*	*0.45*		
R-Squared	0.9846				
Adj R-Squared	0.9736				

ure 1B). Therefore, time is also an important parameter involved throughout the process, besides the temperature, with a crucial influence in ACR formation. ACR was also formed at the lower temperatures (59.3°C and 80°C), but in lower amount (8.49 and 5.48 μg/kg DW, respectively). In a study conducted by Roach *et al.* [2003], the formation of ACR in prune juice was highlighted at a temperature range below 120°C (98–116°C) at higher moisture conditions. Additionally, in a study conducted by Amrein *et al.* [2007], substantial amounts of ACR were found in plums dried at temperatures below 90°C. Furthermore, besides the temperature and time, the starting reactants present in the food matrices are also important in the Maillard reaction, such as sugar and amino acid type [Yaylayan & Stadler, 2005]. Although, the ACR formation involves the condensation of the amino group of asparagine (as the principal precursor) and the carbonyl groups of reducing sugars, when the samples are subjected to heat [Becalski *et al.,* 2003; Mottram *et al.,* 2002; Stadler *et al.,* 2002; Zyzak *et al.,* 2003], other amino acids may have a positive effect in obtaining small amounts of ACR in some model systems, such as proline, tryptophan, cysteine, glycine, lysine, *etc*. [Yu *et al.,* 2013; Koutsidis *et al.,* 2009]. The differences in ACR content between P1 and P2 plum purée at a temperature below 80°C, apart from the variations of heating duration, could also be caused by the higher content of proline in P2 sample, which could lead to a low ACR production. In a study reported by Koutsidis *et al.* [2009], proline and tryptophan (80%) were the most effective amino acids involved in decreasing the ACR levels followed by cysteine and glycine (45–55%). Thermal treatment (temperature above 120°C) can initiate the deamination and decarboxylation of asparagine, with a higher yield of ACR when a carbonyl source is present [Yaylayan *et al.,* 2003; Weisshaar *et al.,* 2002]. This fact can explain the ACR content in P1 and P2 plums, that are rich in reducing sugars and have a high level of asparagine (Table 2). According to Mottram *et al*. [2002], the ACR production is slightly influenced by the presence of glutamine and aspartic acid; the ACR quantities obtained were very low,

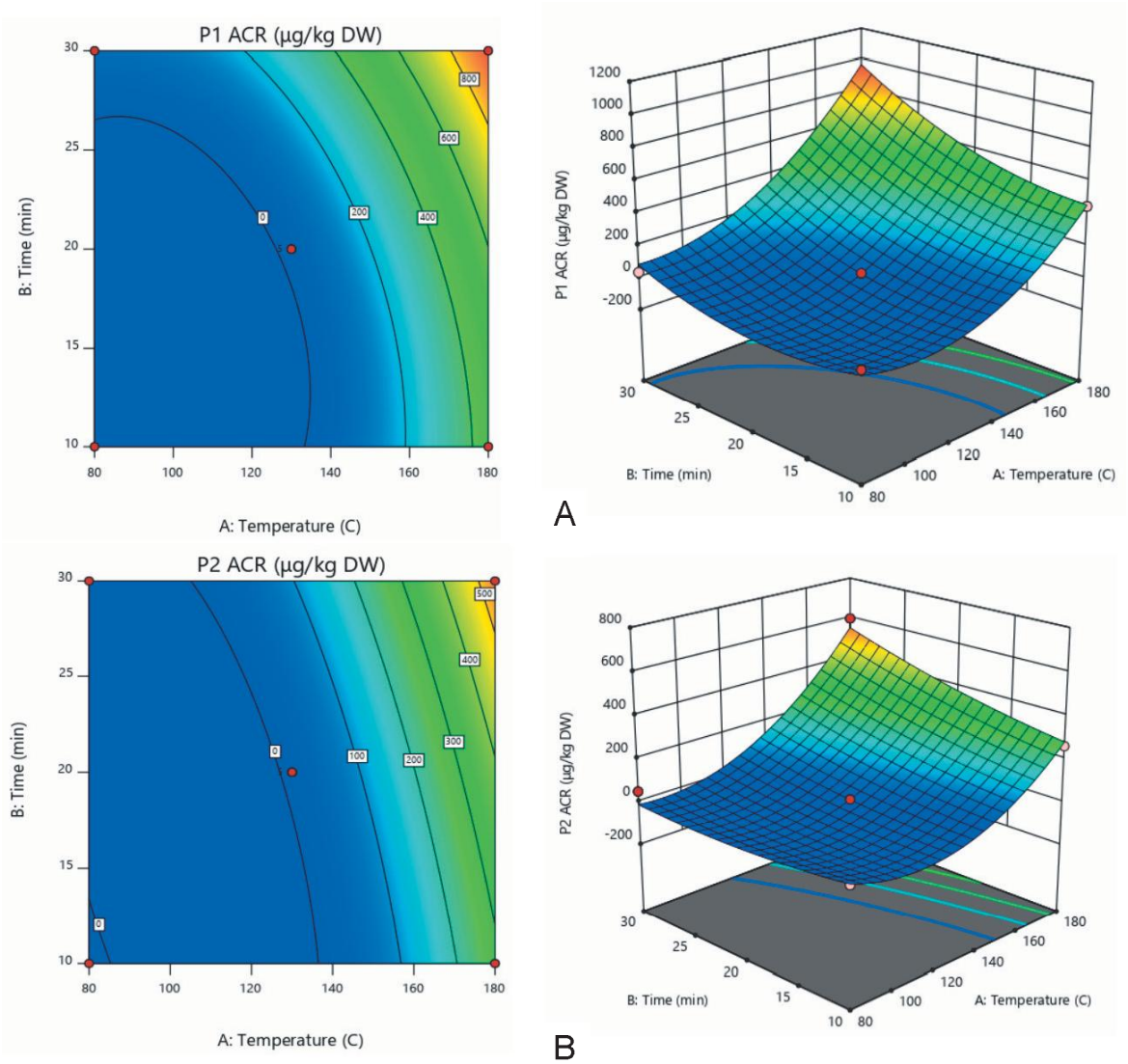

FIGURE 1. Contour graphs describing the quantitative correlative effects of time and temperature on the formation of acrylamide (ACR) in puree of plum *Prunus cerasifera* (P1) (A) and *Prunus domestica* (P2) (B).

in the range of 0.5–1.0 mg/mol. In another study, Zyzak *et al.* [2003] used a system model containing: amino acid, glucose, maltodextrin, emulsifier, potato starch, and water, to identify the ACR formation mechanism. The study revealed that a high quantity of ACR was formed (9270 µg/kg) in the model system with asparagine. Additionally, high quantities of ACR were obtained in the system with glutamine (156 µg/kg) and low quantities (<50 µg/kg) in the systems with: alanine, arginine, aspartic acid, cysteine, lysine, methionine, threonine, and valine. In our study, among the amino acids involved in the ACR formation, the highest content was found for asparagine, however limited quantities of alanine, arginine, aspartic acid, lysine, and valine were found as well (Table 2). There is a few data on the ACR levels in thermally obtained plum products such as jams. In a survey on ACR content in various thermally processed plum products from the Slovak market, the level of ACR varied between 15 µg/kg and 46 µg/kg [Kukurová *et al.,* 2015]. In a similar study regarding the ACR levels in foods from the Turkish market, Ölmez *et al.* [2008] found less than 10 µg/kg ACR in strawberry jam. However, different ACR contents in thermally treated fruits were reported, such as: 14.74–1680 µg/kg in dried plums [Amrein *et al.,* 2007; De Paola *et al.,* 2017], 1432–1502 µg/kg in dark pears, 0–19 µg/kg in pears, 173.43–879.92 mg/kg in Abu variety banana fritter, and 30.07–201.18 mg/kg in Awak variety banana fritter [Daniali *et al.,* 2013].

Regulatory bodies have not established so far the minimum and maximum content of ACR in thermally treated products. Meanwhile, the industrial environment has continuously been concerned to implement measures to reduce the amount of ACR in food based on the application of effective mitigation and quantification strategies. The European Commission recommends the selection of raw material with a reduced level of ACR precursors for the baby jar foods (low-acid and prune-based foods) [Commission Regulation (EU) 2017/2158].

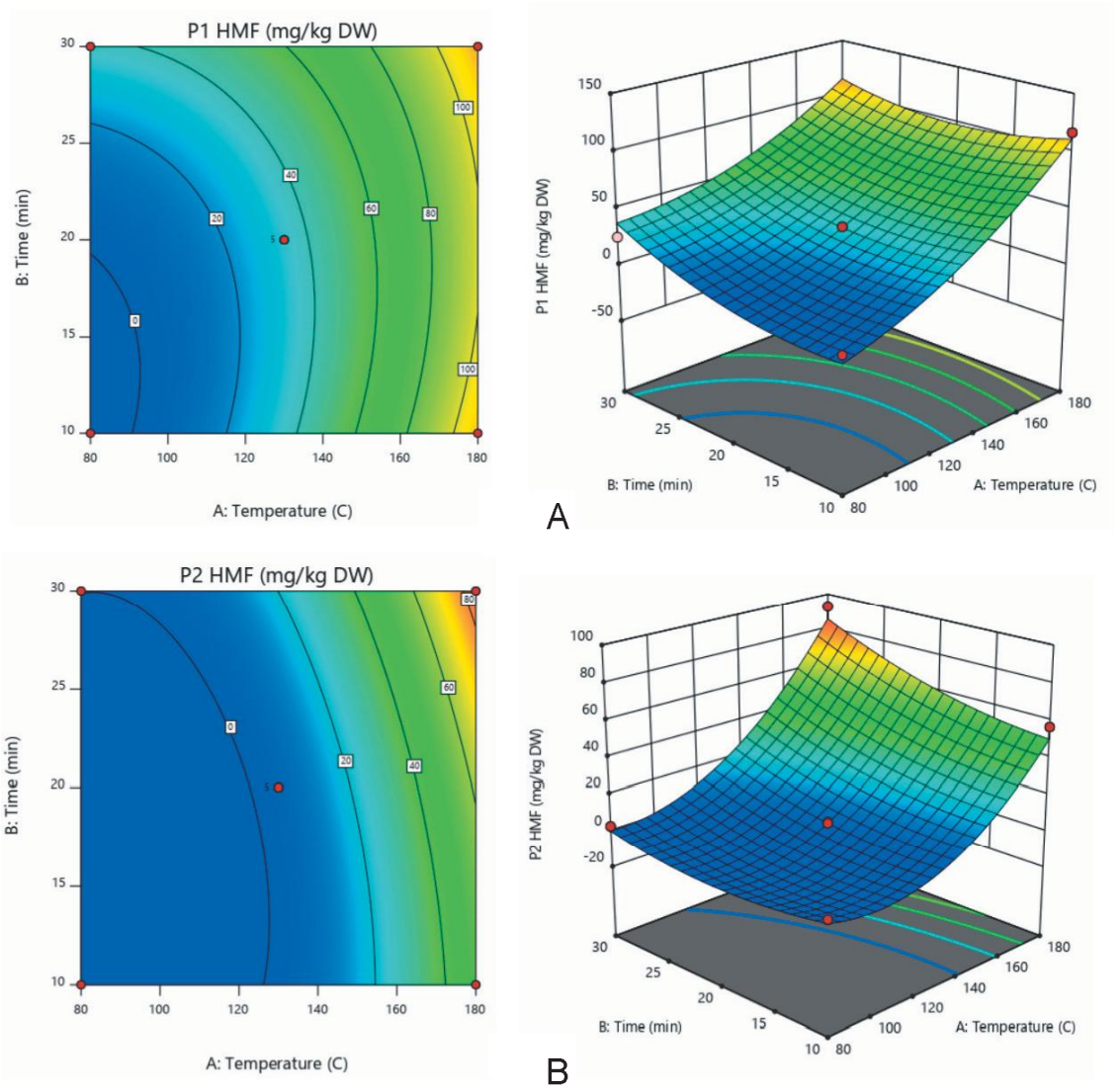

FIGURE 2. Contour graphs describing the quantitative correlative effects of time and temperature on the formation of hydroxymethylfurfural (HMF) in puree of plum *Prunus cerasifera* (P1) (A) and *Prunus domestica* (P2) (B).

Hydroxymethylfurfural formation

As it can be seen in Table 1, a low HMF content was found for variants 3 and 4 of P1 sample and for variants 3, 4 and 8 of P2 sample. By using low temperatures for heat treatment of plums, the amount of HMF formed was lower, even if the time treatment was longer. The CCD allowed estimating equations which enable predicting the most suitable models for the production of HMF for both samples (P1 and P2) as follows (Eq. 4 and 5):

$$P1\ HMF = +33.71 + 48.52\,A + 11.75\,B - 10.30\,AB + 16.31\,A^2 + 14.12\,B^2 \quad (4)$$

$$P2\ HMF = +4.49 + 34.27\,A + 9.15\,B + 8.38\,AB + 22.72\,A^2 + 6.48\,B^2 \quad (5)$$

From the ANOVA (Table 4) for the model chosen, the significant terms are A, B, A^2, and B^2 for plum purée P1 and A, B, AB, B^2 and A^2 for plum purée P2. The HMF content in samples P1 and P2 was positively correlated with all individual terms; the A term (temperature response) having the greatest influence on HMF formation. In the case of P1 sample, HMF content was negatively correlated with AB. Figure 2 (A, B) depicts the temperature-time effect on HMF production. HMF formation was minimal at exposure time between 10 and 25 min, and at exposure temperature of around 120°C. HMF is formed by heating, as an intermediate in the Maillard reaction, [Mauron, 1981; Glatt & Sommer, 2006]. Kavousi *et al.* [2015] suggested that the presence of the amino acids, glutamine, glutamic and aspartic acids led to an accelerated formation of HMF compared with the addition of basic amino acids in the model systems. Another mechanism for HMF formation implies direct thermal dehydration of fructose, sucrose, and glucose, without the presence of amino groups [Antal *et al.,* 1990].

By analyzing the 13 running variants, the highest level of HMF was obtained in the plum purée exposed to 200°C for 20 min for P1 plums (139.06 mg/kg DW) and to 180°C for 30 min for P2 plums (92.88 mg/kg DW) (Table 1). According

to Kocadagli *et al.* [2012], chlorogenic acid promotes the hydrolysis of sucrose to fructose, at a temperature above 180°C, that may contribute to HMF formation. Moreover, in a study conducted by Zhang *et al.* [2016] by heating fructose with or without aspartic acid (at 90°C /48 h), to simulate plums drying, it was found that chlorogenic acid increased HMF concentration. In this case, the tested temperature was below 100°C, but the time of exposure was longer.

The amino acids present in the food matrix may also contribute to the increase of HMF content, probably due to the sucrose hydrolysis which is catalyzed by the presence of amino acids [Lee & Nagy, 1990]. Moreover, a higher content of HMF was formed by heating fructose with aspartic acid at pH 7.0, compared to fructose alone [Zhang et al., 2016]. In a study conducted by Rada-Mendoza *et al.* [2002] where HMF was analyzed in various types of fruit jams (apple, apricot, banana, bilberry, fig, lemon, mulberry, orange, pineapple, plum, strawberry), the obtained results varied between5.5 and 37.7 mg/kg. In our study, the HMF content varied as a function of temperature/time coordinates between 0.25 and 139.06 mg/kg in P1, and between 0.18 and -92.88 mg/kg in P2, respectively.

The HMF levels in food are established only for honey by Codex Alimentarius, that allows a maximum concentration of 40 mg HMF /kg of productand also by the industry that set an upper limit for fruit juice at 20 mg/kg [Morales *et al.,* 2008]. For plum products and jams, the regulatory bodies have not set a limit for HMF content.

Global optimization

The "Desirability" approach is one of the most widely used methods in the industry for optimizing multiple response processes. The global desirability function is defined as the geometric mean of the partial functions. A non-zero value of desirability implies that all the selected criteria were in a good combination and a value closest to 1 shows the best of combinations. Therefore, for sample P1 a 0.895 value of Desirability was obtained, that implied value of 27.8 mg/kg DW for HMF and a value of 3.90 μg/kg DW for ACR. For sample P2, similar results were obtained, such as a 0.979 value of Desirability which correlates to a minimum of 3.95 mg/kg DW for HMF and 8.74 μg/kg DW for ACR.

CONCLUSIONS

Our study data suggest that ACR is formed in the heat-treated plum purée samples through the specific amino acid pathway and is due to the high contents of reducing sugars and asparagine. In general, the contents of ACR and HMF in the tested plum purées highly depended on the applied combination of time and temperature, with a minimum ACR content obtained between 10 and 20 min and an increase in contents of both tested toxicants at higher temperature.

Central Composite Design model was used to estimate equations predicting the most suitable models for the production of ACR and HMF in the treated samples. The global desirability of the both selected varieties were close to 1, showing the best of combinations. Therefore, the optimal parameters in terms of temperatures and time combination that should be applied are: 114.2°C/23.8 min for *Prunus cerasifera*, and 127.7°C/21 min for *Prunus domestica*.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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