EFFECT OF HOP EXTRACT ADDITION ON HETEROCYCLIC AROMATIC AMINES FORMATION IN BEEF PATTIES

T. Polak[1], M. L. Polak[1], M. Malenšek[1] and L. Demšar[1]

[1] Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Jamnikarjeva 101, Slovenia

Abstract – The effect of different Slovenian hops' extracts containing xanthohumol on the HAAs formation in beef patties after thermal treatment was investigated. Ethanolic extracts were prepared from four different hop varieties (Aurora, Bobek, Celeia and Savinjski golding) and then bound to salt. Two different amounts (0.5 g and 1.0 g) of these extracts were added into beef patties. After thermal treatment, heterocyclic aromatic amines (HAAs) were determined by LC-MS. The results obtained show that addition of both amounts of different ethanolic hop extracts (bound on salt) reduced the HAAs formation in beef patties.

Key words – xanthohumol, Slovenian hop variety, salt bound extract

I. INTRODUCTION

Heterocyclic aromatic amines (HAAs), considered as possible human carcinogens, are a group of 20 different chemical compounds formed in cooked meats (above 200 °C) through pyrolysis reactions of different amino acids in the presence or absence of creatine/creatinine and sugars [1]. The extent to which they are produced depends upon type of meat, cooking temperature, cooking length and degree of browning during cooking [2, 3]. Studies revealed that grilled, fried, barbecued, broiled and roasted meat samples show high amounts of HAAs [4]. Antioxidants are considered to be beneficial for the HAAs elimination [5]. An example of compounds exhibiting antioxidative activity is a group of polyphenols. They are widely distributed in plants. Female hop flowers used as flavoring agent providing beer bitterness contain several important polyphenolic compounds, among which xanthohumol, a prenylated chalcone, should be mentioned [6].

The objective of this study was to evaluate the effect of different Slovenian hop extracts containing xanthohumol on the HAAs formation in beef patties after thermal treatment.

II. MATERIALS AND METHODS

Hop extracts were prepared from four different Slovenian hop varieties of year 2013: Aurora, Bobek, Celeia and Savinjski golding.

Previously homogenized dry hop cones were weighted (10 g) in beakers, and 200 mL 70% ethanol was added. This was followed by stirring on a magnetic stirrer. The samples were then transferred into 50 mL centrifuge tubes and centrifuged for about 5 minutes. The supernatant was filtered through a filter paper in a 200 mL volumetric flask and filled up to the mark with ethanol. The ethanolic extracts were stored in the refrigerator.

To bind the ethanolic hop extracts to salt, 10 g table salt was weighted into plastic container. 10 mL ethanol hop extract was added, mixed well and dried overnight in an oven at 60 °C. After drying, the salt was triturated and stored in plastic containers.

The patties (80 g weight) were prepared from minced beef. Two different amounts (0.5 and 1.0 g) of salt bound hop extract were added. Samples of patties without addition of hop extract were used as a control. Petri dishes formed patties were grilled in a Teflon-coated pan for 4 min at 240 °C, weighed, packed in polyethylene bags, frozen and then ground in a mill to a homogeneous structure.
For xanthohumol determination in ethanolic hop extracts solid phase extraction was performed using Strata-X 33u polymeric Reversed Phase column (Phenomenex). The pre-conditioning was carried out with 8 mL methanol and 8 mL distilled water. Each sample (prepared from 1 mL of ethanol hop extract and 5 mL of distilled water)

was slowly and quantitatively transferred onto a column. The column was washed with 8 mL of distilled water and followed by 5 min of drying. The elution was carried out with 8 mL methanol:acetonitrile (v/v, 1:1). The eluate was collected in glass vials. Thus prepared, the samples were kept frozen at –20 °C until LC-MS analysis.

The residues of heterocyclic aromatic amines (HAAs) were determined by the method described by Santos *et al.* [7], with some modifications. Briefly, the homogenized samples were weighed (3 g) in beakers, dissolved in 12 mL 1M NaOH and spiked with 100 μL 2-amino-3,4,7,8-tetramethylimidazo[4,5-f]quinoxaline (TriMeIOx) in methanol as an internal standard. The suspension was homogenized by magnetic stirring for 12 h at 500 rpm. This alkaline solution was mixed with diatomaceous earth (12 g) and transferred to empty chromatographic columns with frits. 70 mL ethyl acetate was poured onto a glass column; the sample was loaded and slowly extracted. For LC-MS analysis, the clean-up was performed by the solid phase extraction, using Oasis, MCX 60 mg columns. The pre-conditioning was carried out with 2 mL methanol and 2 mL ethyl acetate. Eluate was slowly and quantitatively transferred onto a column and then washed with 2 mL 0.1 M HCl solution and 2 mL methanol. The column was dried off. The elution was carried out with 2 mL methanol:ammonia (v/v, 19:1). The eluate was collected in glass vials. The solvent was evaporated to dryness under a stream of nitrogen, and the final extracts were dissolved in 250 mg methanol just before measurement.

The LC-MS analyses were performed on an Agilent 1100 system. The analytical column was operated under reverse phase (Semi Micro TSKgel ODS-80Ts column, 5 μm, and 250 mm × 2 mm i.d.) from Tosoh Bioscience LLC (Japan, 18151) at a temperature of 25 °C. The separation was performed at a flow rate of 0.300 mL min^{-1} by gradient elution with 20 mmol L^{-1} ammonium formate (Fluka, 09739) at pH 3.2 as solvent A, and acetonitrile (Merck, 1.00030) as solvent B. The gradient program was: 95% A, 0.0–0.5 min; 95–80% A, 0.5–15.0 min; 80–40% A, 15.0–18.0 min; 40% A, 18.0–24.0 min; 40–95% A, 24.0–27.0 min; 95% A, 27.0–40.0 min. An injection volume of 10 μL was used, with the internal standard of 4,7,8-TriMeIQx. The HAAs were identified and quantified using retention times and the spectra from reference samples of known concentrations, run under the same conditions. The following HAAs were quantified: Harman Cat. No. H105000, Norharman Cat. No. N700000, IQ Cat. No. A616500, MeIQ Cat. No. A605200, IQx Cat. No. A616900, MeIQx Cat. No. A606600, 4,8-DiMeIQx Cat. No. A631000, 4,7,8-TriMeIQx Cat. No. A630000, and PhIP Cat. No. A617000, all obtained from Toronto Research Chemicals.

III. RESULTS AND DISCUSSION

The highest content of xanthohumol in hop samples was determined in the Aurora variety (3.12 mg g^{-1}) and the lowest in the Celeia variety (1.29 mg g^{-1}) (Table 1).

Table 1: The content of xanthohumol in hop (mg g^{-1}) and in ethanolic hop extracts (mg mL^{-1})

Varieties	Aurora	Bobek	Celeia	S. golding
Xanthohumol (mg g^{-1})	3.12	2.52	1.29	1.39
Xanthohumol (mg mL^{-1})	0.16	0.13	0.06	0.07

S. golding – Savinjski golding

Similar xanthohumol amounts were identified in Slovenian hop varieties in 2005 by Hrastar *et al.* [8]. Their results confirm the impact of the variety on xanthohumol content. Savinjski golding and Celeia both contain from 2.3 to 2.7 times less xanthohumol than Aurora variety. The highest xanthohumol concentrations in ethanolic extracts were determined in Aurora (0.16 mg mL^{-1}) and Bobek (0.13 mg mL^{-1}) varieties.

The data obtained show that the addition of different ethanolic salt bound hop extracts affects (both amounts, 0.5 g and 1.0 g) the total HAAs formation after thermal treatment in beef patties (Table 2). More effective total HAAs formation inhibition was observed at larger amount of added extract (1 g): 80.7% for Bobek, 76.2% for Celeia and 76.5% for Savinjski golding variety, compared to the control (no hop extract addition), respectively. In general, with increasing xanthohumol concentration the HAAs content in beef patties decreases. The exception was the addition of 0.5 g extract of Aurora variety, where

the content of total HAAs was reduced for 72.9%, in comparison with addition of 1 g extract where the content of HAAs was reduced for 56.7%. Among individual HAAs the PhIP and Harman contents should be mentioned - both were significantly degraded at both amounts of all hop extracts varieties. The maximum reduction of PhIP (95.5%) and Harman (95.5%) occurred at 1 g ethanolic salt bound hop extract addition prepared from Savinjski golding variety. The content of Norharman, Glu-P-2 and MeIQx were also decreased (compared to control), but there were no significant differences established between added extracts amounts. Hop extracts did not statistically affect the formation of IQ and IQx. We have not been able to find similar studies concerning the influence of xanthohumol on HAAs formation, which makes any specific comparisons with our data difficult. There are many investigations available about polyphenolic compounds and their effect on the HAAs formation in different foods.

Table 2: The effects of the hop varieties and amount of added ethanolic salt bound hop extracts (g) on content (mg kg^{-1} of patty) of individual and total HAAs in patties after thermal treatment (Duncan test, $\alpha = 0.05$)

HAA	c	Aurora	Bobek	Celeia	S. golding
Norh.	0.0	8.07±1.16	8.07±1.16	$8.07±1.16^{A}$	$8.07±1.16^{A}$
	0.5	6.03±1.05	4.91±4.12	$5.67±1.88^{AB}$	$4.46±0.89^{B}$
	1.0	$7.41±0.29^{a}$	$2.71±2.16^{b}$	$2.37±2.16^{bB}$	$1.23±0.15^{bC}$
Harman	0.0	$24.51±4.48^{A}$	$24.51±4.48^{A}$	$24.51±4.48^{A}$	$24.51±4.48^{A}$
	0.5	$4.42±0.60^{bB}$	$5.99±0.38^{bB}$	$5.93±0.32^{bB}$	$9.05±1.91^{aB}$
	1.0	$4.75±0.48^{B}$	$1.86±1.38^{B}$	$5.32±7.44^{B}$	$1.11±0.15^{C}$
Glu-P-2	0.0	$51.05±28.12^{A}$	$51.05±28.12^{A}$	$51.05±28.12^{A}$	51.05±28.12
	0.5	$0.72±0.63^{bB}$	$2.58±0.37^{bB}$	$7.14±6.43^{bB}$	$19.51±2.27^{a}$
	1.0	$10.94±4.93^{bB}$	$8.76±5.46^{bB}$	$16.10±6.17^{abB}$	$24.29±6.40^{a}$
IQ	0.0	1.70±2.94	1.70±2.94	1.70±2.94	1.70±2.94
	0.5	0.68±0.42	0.37±0.32	1.48±1.09	1.22±1.11
	1.0	0.25±0.22	0.32±0.30	0.13±0.11	1.70±1.53
IQx	0.0	$<0.01^{B}$	$<0.01^{B}$	$<0.01^{B}$	$<0.01^{B}$
	0.5	$10.42±2.92^{A}$	$10.75±1.55^{A}$	$9.87±4.00^{A}$	$6.51±1.30^{A}$
	1.0	$13.22±1.03^{aA}$	$4.22±7.31^{bAB}$	$1.06±1.83^{bB}$	$<0.001^{bB}$
MeIQx	0.0	$25.67±5.17^{A}$	$25.67±5.17^{A}$	$25.67±5.17^{A}$	$25.67±5.17^{A}$
	0.5	$8.18±3.37^{B}$	$9.93±1.03^{B}$	$13.64±3.77^{B}$	$13.25±3.67^{B}$
	1.0	$20.74±1.49^{aA}$	$7.26±6.34^{bB}$	$6.39±2.26^{bB}$	$3.60±2.45^{bC}$
PhIP	0.0	$27.46±1.65^{A}$	$27.46±1.65^{A}$	$27.46±1.65^{A}$	$27.46±1.65^{A}$
	0.5	$7.07±1.49^{bB}$	$11.28±0.73^{abB}$	$14.05±3.98^{aB}$	$12.76±2.97^{aB}$
	1.0	$2.70±0.17^{aC}$	$1.55±1.10^{abC}$	$1.56±0.82^{abC}$	$0.70±0.18^{bC}$
Total	0.0	$138.5±19.4^{A}$	$138.5±19.4^{A}$	$138.5±19.5^{A}$	$138.5±19.5^{A}$
	0.5	$37.5±6.6^{cB}$	$45.8±6.3^{bcB}$	$57.8±4.63^{abB}$	$65.8±10.7^{aB}$
	1.0	$60.0±6.6^{aB}$	$26.6±17.7^{bB}$	$32.9±17.9^{bB}$	$32.6±7.8^{bC}$

c - amount of ethanolic salt bound hop extracts in g, Norh - Norharman, S. golding – Savinjski golding, means with a different superscript within rows ($^{A, B, C}$) per parameter differ significantly ($P \leq 0.05$, significance of differences between hop varieties); means with a different superscript within columns ($^{a, b, c}$) differ significantly ($P \leq 0.05$, significance of differences between amount of added ethanol hop extract bound on salt)

For example, Balogh *et al.* [9] evaluated that the rosemary extract addition (1 and 10%) in beef patties reduced the total HAAs content (IQ, MeIQx, 4,8-DiMeIQx and PhIP) for 51.5%, respectively.

IV. CONCLUSION

In the present study we confirmed that xanthohumol addition from ethanolic salt bound extracts in different Slovenian hop varieties reduced the formation of HAAs in beef patties. Further investigations are needed for better understanding of the xanthohumol inhibition effect.

ACKNOWLEDGEMENTS

Authors of this paper thank the company Hmeljarstvo Čas/Lupoin d.d. for different Slovenian hop varieties.

REFERENCES

1. Sugimura, T. (1997). Overview of carcinogenic heterocyclic amines. Mutation Research 376: 211-219.
2. Skog, K.I., Johansson, M.A., Jagerstad, M.I. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. Food and Chemical Toxicology 36: 879-896.
3. Cross, A. J., Sinha, R. (2004). Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environmental and Molecular Mutagenesis 44: 44-45.
4. Rahman, U., Sahar, A., Khan, M.I., Nadeem, M. (2014). Production of heterocyclic aromatic amines in meat: Chemistry, health risks and inhibition. Food Science and Technology 59: 229-233.
5. Hala, A. (2011). The effect of green tea and olive oil on the mutagenic activity of heterocyclic amines extracted from common food consumed in Saudi Arabia. International Journal of Food Science and Nutrition 62: 295-302.

6. Jirasko, R., Holčapek, M., Vrublova, E., Ulrichova, J., Šimanek, V. (2010). Identification of new phase II metabolites of xanthohumol in rat in vivo biotransformation of hop extracts using high-performance liquid chromatography electrospray ionization tandem mass spectrometry. Journal of Chromatography 1217: 4100-4108.

7. Santos, F.J., Barcélo-Barrachina, E., Toribio, F., Puignou, L., Galceran, M.T., Persson, E., Skog, K., Messner, C., Murkovic, M., Nabinger, U., Ristic, A. (2004). Analysis of heterocyclic amines in food products: interlaboratory studies. Journal of Chromatography 802: 69-78.

8. Hrastar, R., Kač, M., Košir, I.J. (2006). Vpliv sorte in lokacije na vsebnost ksantohumola v hmelju. Hmeljarski bilten 13: 5-13.

9. Balogh, Z., Gray, J.I., Gomaa, E.A., Booren, A.M. (2000). Formation and inhibition of heterocyclic aromatic amines in fried ground beef patties. Food and Chemical Toxicology 38: 395-401.