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Onion waste as a rich source of antioxidants for meat products

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Abstract: Onion skin is a waste produced in thousands of tons annually in the European Union. It is a rich natural source of flavonoids and its water extracts (as an environmentally friendly solvent) could be used as an antioxidant material for meat products. Therefore, antioxidant properties of onion skin water extracts (OSWEs) were tested on cooked pork patties. Pork patties were divided into five treatments: control (no antioxidant), 10 and 20% (w/w) of yellow OSWE, and 10 and 20% (w/w) of red OSWE. Antioxidant activity, total polyphenols, thiobarbituric acid reactive substances (TBARS), and sensory analysis were assessed. Patties with added antioxidants showed significantly ($P < 0.05$) higher antioxidant activity and total polyphenol content. Samples with OSWEs, after 5-day storage (5°C), had significantly ($P < 0.05$) lower TBARS values compared to control. Two main phenolic compounds were identified in OSWEs by liquid chromatography – mass spectrometry using electrospray ionisation in negative mode: quercetin (*m/z* 301) and quercetin monoglucoside (*m/z* 463). OSWEs demonstrated the potential to be used as a source of natural antioxidants with strong antioxidant activity in meat products.

Keywords: antioxidant; LC/MS; onion skin; pork patties

Natural materials and ingredients are increasingly more preferred by consumers because of health concerns, thus the utilization of them is an emerging field in food science. Onion (*Allium cepa* L.) is one of the most common species of vegetable in the world, and it is a very important source of flavonoids in the

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human diet (Beesk *et al*. 2010). Thousands of tons of industrial onion waste are produced annually in the European Union (Kiassos *et al*. 2009). Certain parts of onion waste are rich in flavonoids with the richest being onion skin, where the most abundant compound with antioxidant and radical scavenging properties is quercetin and its glycosides (Albishi *et al*. 2013). These phenolics could reduce some unwanted processes in foods.

Lipid oxidation is one of the most unfavorable chemical reactions that takes place in meat products (Rohlík *et al*. 2013), which can lead to a deterioration of the sensory and nutritional parameters of meat products (Nuñez de Gonzalez *et al*. 2008). The addition of antioxidants can effectively inhibit or prevent these undesired processes (Šojić *et al*. 2017). Previous research shows that ethanol onion skin extracts, as a natural antioxidant source, can reduce meat lipid oxidation (Shim *et al*. 2012) and can also eliminate microbial spoilage (Alahakoon *et al*. 2013). There are many conventional extraction methods using organic solvents (e.g. maceration, sonication and soxhlet) and some newer 'green extraction techniques' such as pressurized liquid extraction, supercritical CO_2 extraction and microwave-assisted extraction suitable for obtaining antioxidants from plant material (Campone *et al*. 2018), however, these procedures require specific instruments.

Water, unlike ethanol, is an environmentally friendly solvent without any negative impact on consumer health, and it is also the only liquid used in food preparation in amounts up to 30% (for example in some recipes of meat products). To the best of our knowledge, there is no data in scientific literature that deals with the effects of onion skin water extracts on meat product quality.

Based on all arguments mentioned above, our aim was to evaluate antioxidant activity, total polyphenol content, lipid peroxidation and sensory attributes of meat products fortified with natural antioxidants obtained from industrial onion waste, using water as extraction agent. Additionally, high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) analysis was conducted to determine main phenolic antioxidants in onion waste extracts.

MATERIAL AND METHODS

Material and chemicals. Fresh pork neck meat was obtained from the local retail market in Prague, Czech Republic. Meat was stored at 5°C in the dark, approximately 10 h before use. Dry onion skin as a waste was obtained directly from the grower (Vital Czech s.r.o., Czech Republic). For this experiment, two colours of onion skin were used, red (var. Lisa) and yellow (var. Hybelle).

All chemicals, namely Trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid, Folin-Ciocalteu reagent, butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), gallic acid, 1,1,3,3-tetraethoxypropane (TEP), *ortho*-phosphoric acid (H_3PO_4), sodium carbonate, acetonitrile (LiChrosolv, LC/MS grade), formic acid (reagent grade), quercetin dihydrate and quercetin-3,4´-diglucoside were purchased from Sigma-Aldrich (Czech Republic).

Preparation of onion skin water extracts (OSWEs). Two variants of OSWE were prepared, one was from yellow onion skin (YWE – yellow water extract) and one from red onion skin (RWE – red water extract). The onion skins were washed to remove residues of soil and then dried in an oven (Memmert UN 75; Memmert, Germany) for 8 h at 40°C. Washed and dried onion skins (50 g) were added to 2 l of water. This mixture was gently boiled (100°C) for 40 minutes. Thereafter, the mixture was left for 15 min to cool down and then filtered. The onion skin was then manually squeezed to obtain remaining liquid extract. OSWEs were immediately used for pork patty preparation, HPLC-ESI-MS/MS analysis, determination of antioxidant activity (AOA) and also for total polyphenolic content determination (TPC).

Preparation of pork meat patties. Approximately 3 kg of pork neck meat was minced using a 2 mm plate. After mincing, the meat was divided into one of the following five treatments: control (no antioxidant); 10Y (10% w/w of YWE); 10R (10% w/w of RWE); 20Y (20% w/w of YWE) and 20R (20% w/w of RWE). Also, 2% (w/w) of salt was added to all samples. When all ingredients were added, the meat was properly hand-mixed and then formed into patties (50 g portions). A hot air oven (T-fal Maxi-Oven Varmluft; Tefal, France) was used to cook the patties for 17 min at 200°C to reach an internal temperature of 70°C for 10 minutes. After 30 min, when the temperature of the patties decreased to room temperature, they were aerobically packed into low density polyethylene bags and stored at 5°C for 5 days and analysed for TPC, AOA, organoleptic properties, and thiobarbituric acid reactive substances (TBARS). TPC, AOA, dry matter and fat content were analysed before and after the heat treatment, and TBARS after cooking

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(day 0) and after 5 days of storage. Sensory analysis was conducted after cooking at day 0.

Qualitative analysis of main antioxidant compounds in OSWEs by HPLC-ESI-MS/MS. Firstly, extraction was performed according to Cheng *et al*. (2013) with modifications. Briefly, 0.25 g of onion skin water extracts (OSWE) was dissolved in 12 ml of methanol/water (80:20, v/v). The mixture was sonicated for 1 h at room temperature and then centrifuged at 4000 rpm for 15 minutes. Extracts were filtered through a 0.45 µm glass filter and for the analysis 20 µl (diluted if needed) was injected into an HPLC system.

Analysis of the OSWEs was accomplished using a Dionex UltiMate 3000 UHPLC system (Dionex, Germany), which consisted of a quaternary pump, column compartment, and UV detector. Separation was performed on a Phenomenex Kinetex C18 100Å analytical column (150 mm × 2.1 mm, 2.6 µm,). Column temperature was maintained at 35°C. A linear gradient elution with mobile phase A (5% acetonitrile + 0.5% formic acid in water) and B (100% acetonitrile) was used as follows: mobile phase B increased from 5% to 60% within 35 min, then decreased back to 5% in 5 min to equilibrate the system. Flow rate was 0.2 ml/min and analytes were detected at 355 nm.

After separation in the HPLC system, qualitative analysis was conducted on an Agilent 6420 Triple Quadrupole Mass Spectrometer (Agilent Technologies, USA). The mass spectrometer was operating in negative electrospray ionization (ESI) mode. For drying and nebulising nitrogen (N_2) gas was used at a flow rate of 11 l/min and nebulising pressure of 15 psi. Drying gas temperature was set at 300°C and capillary voltage at 4000 V. Fragmentor, collision, and cell accelerator voltages were 100, 10 and 7 volts, respectively.

Antioxidant activity (DPPH assay). The free radical-scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to Brand-Wiliams *et al*. (1995) and Sanchez-Moreno *et al*. (1998). The antioxidant activity was related to Trolox and expressed as mg of Trolox equivalent (TE) per g of dry weight (DW).

Determination of total phenolic content (TPC). The amount of total phenolics was determined using Folin-Ciocalteau reagent (Lachman *et al*. 1997) and calculated as gallic acid equivalent (GAE) in mg per g of DW.

Determination of dry matter and fat content. Patties were analysed for its dry matter (ISO 1442:1997) and total fat content (AOAC 922:06) and expressed as a mass percentage of fresh weight (Table 1).

Thiobarbituric acid reactive substances (TBARS). For expression of lipid oxidation, TBARS assay was used according to Miller (1998) with modifications. To 1 g of homogenized sample, 0.2 ml BHT (0.2 mg per ml in methanol) and 9.1 ml 10% TCA in 0.2M H_3PO_4 solutions were added and homogenized with T18 Basic Ultra-Turrax (IKA, Germany) for 1 minute. Homogenate was filtrated and 1.5 ml was transferred to a test tube, then 1.5 ml of 0.02M TBA was added and the mixture was vortexed and heated for 30 min at 85°C. After heating, each sample was pipetted in triplicate on 96 well plates and absorbance was read at 530 nm on Tecan (Eppendorf, Czech Republic). TEP was used as a standard and the results were expressed as µg of malondialdehyde (MDA) per gram of sample.

Sensory analysis. Sensory evaluation of the meat products was conducted in the laboratory of sensory analysis in Food Research Institute, Prague, equipped according to the international standard ISO 8589. The sensory analysis was carried out under ISO 6658 conditions. The assessor panel was composed of 10 highly trained panelists according to ISO 8586. For this evaluation method, 100 mm long non-structured

Table 1. Dry matter and fat content in a raw and cooked patties (%)

Meat patty	Dry matter		Fat content	
	raw	cooked	raw	cooked
Control	32.65 ± 0.76^{aB}	44.11 ± 0.55^{aA}	14.15 ± 0.35^{aB}	19.76 ± 0.39^{aA}
10Y	31.02 ± 0.86^{aB}	43.08 ± 0.06^{aA}	12.21 ± 0.31^{bB}	17.78 ± 0.33^{bA}
10R	30.62 ± 0.93^{aB}	40.95 ± 0.36^{abA}	11.64 ± 0.27^{bB}	17.28 ± 0.33^{bA}
20Y	25.44 ± 0.67^{bB}	42.59 ± 0.29^{abA}	8.84 ± 0.59^{cB}	15.62 ± 1.07^{cA}
20R	26.41 ± 0.28^{bB}	36.08 ± 0.04^{bA}	8.74 ± 0.28^{cB}	15.12 ± 0.27^{cA}

$^{a-c}$means within a column with the same letter do not differ significantly ($P > 0.05$); $^{A–B}$means within a row with the same letter do not differ significantly ($P > 0.05$); control – no antioxidant; 10Y–20Y – meat patty with 10–20% of yellow onion skin water extract; 10R–20R – meat patty with 10–20% of red onion skin water extract

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abscissas were used while panelists recorded 6 attributes: appearance, colour, odour, flavour, texture and overall acceptability (100 = excellent, 0 = very poor).

Data analysis. All analyses were done in triplicate and the results were reported as mean ± standard error. Students' *t*-test, analysis of variance with Tukey's HSD test were used to determine statistically significant differences ($P < 0.05$). Normality of the input data obtained from sensory analysis was checked using the Shapiro-Wilk test and outliers were determined by the Dean-Dixon test. STATVYD Version 2.0 (TBU, Czech Republic) and Statistica 12 (StatSoft, USA) was used for data processing.

RESULTS AND DISCUSSION

Qualitative analysis of main antioxidant compounds in OSWEs by HPLC-ESI-MS/MS. To identify main phenolic compounds in OSWEs, LC-MS analysis was used. Comparison of retention times and mass spectra with those obtained from analysis of available standards and also reports in existing literature were used for compound identification.

It is well known that onion skin contains high amounts of flavonol antioxidants, which are represented mainly by quercetin aglycone and its glucosides (Suh *et al*. 1999). However, our aim was to identify phenolic compounds that are presented in water extracts, since water is not the strongest solvent for less polar compounds such as quercetin (Xu *et al*. 2006).

As illustrated in Figure 1, there are only two main peaks with retention times 14.6 and 18.0 min (peak 2 and 3, respectively) which have been later determined in MS^2 analysis. The fragmentation pattern in negative ionization mode provided very useful information for identification of flavonols. Peak 2 was assigned as quercetin monoglucoside (Figure 2A) which showed fragmentation of the molecular ion as a precursor ion at *m/z* 463 $[M-H]^-$ to fragment ion at *m/z* 301 $[M-162]^-$. The fragment with *m/z* 162 is anhydroglucose and it is typical for the fragmentation of glucosides, which is the same fragmentation as described by Bonaccorsi *et al*. (2008). Peak 3 with a molecular ion at *m/z* 301 $[M-H]^-$ (Figure 2B) was identified as quercetin due to its MS^2 fragments (*m/z* 179 and 151), which are typical for quercetin (Fabre *et al*. 2001).

Also, quercetin 3,4´-diglucoside (peak 1) was identified according to its fragmentation pattern and coelution with authentic standard. The molecular ion at *m/z* 625 $[M-H]^-$ (Figure 2C) showed fragmentation in MS^2 analysis (*m/z* 463 and 301) after losing glucosyl moieties $[M-H-162-162]^-$, which is typical for this compound (Mullen *et al*. 2003). It is one of the most dominant flavonol in edible parts of the onion (Roldán-Marín *et al*. 2009), but in OSWEs it is presented only as minor compound.

Qualitative composition of the main polyphenolic compounds in OSWE corresponds with the composition of onion skin as reported by Kim and Kim (2006), where these authors found only two main peaks (quercetin monoglucoside and quercetin).

AOA and TPC content in OSWEs and meat patties. The results of AOA and TPC assays in OSWEs are presented in Table 2. The RWE had significantly higher ($P < 0.05$) antioxidant activity and total polyphenol content than YWE. This confirms that red skin onions are a richer source of antioxidants than yellow

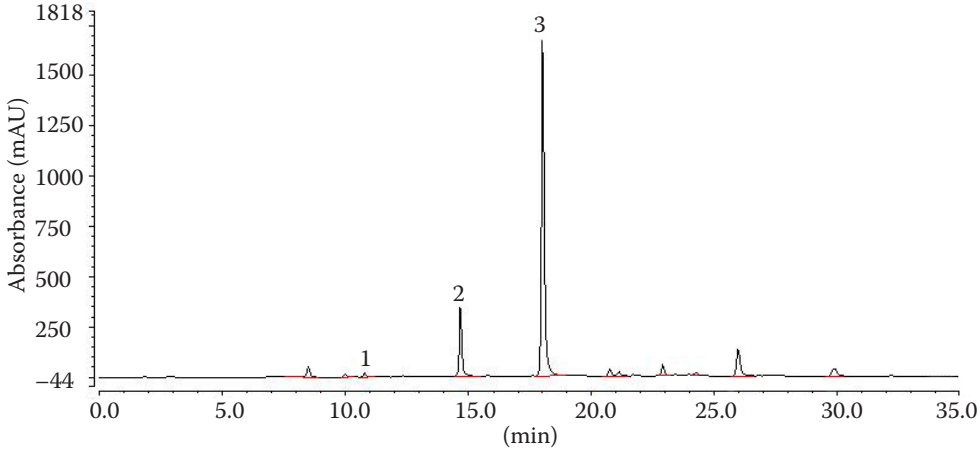

Figure 1. Typical HPLC chromatogram of OSWE recorded at 355 nm
1 – quercetin-3,4´-diglucoside, 2 – quercetin monoglucoside, 3 – quercetin

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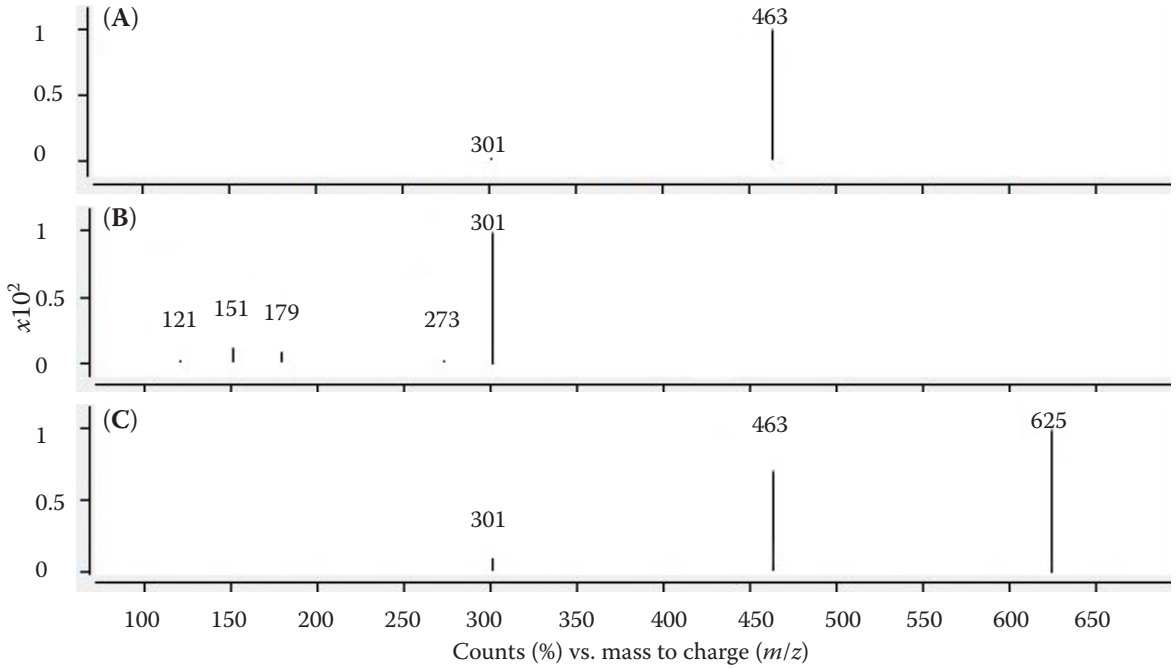

Figure 2. Mass spectrum (ESI in negative mode) of selected main and minor constituents of OSWE: (**A**) quercetin monoglucoside; (**B**) quercetin; (**C**) quercetin 3,4´-diglucoside

varieties, which is in accordance with the findings of many authors (Albishi *et al*. 2013; Lee *et al*. 2015; Ren *et al*. 2017). A positive linear correlation (R^2 = 0.998; graph not shown) between antioxidant activity and total polyphenol content in OSWEs was also found, which suggests that the polyphenolic compounds contributed significantly to the antioxidant activity of OSWEs. This correlation corresponds with other authors which reported similar results (Cheng *et al*. 2013).

Table 2. Antioxidant activity (DPPH) and total polyphenols content (TPC)

Sample	DPPH (mg TE/g FW)	TPC (mg GAE/g FW)
YWE	3.46 ± 0.06^{b}	1.99 ± 0.03^{b}
RWE	6.35 ± 0.32^{a}	4.13 ± 0.25^{a}

$^{a–b}$means within a column with the same letter do not differ significantly (P > 0.05); FW – fresh weight; YWE – yellow onion skin water extract; RWE – red onion skin water extract

AOA and TPC in pork meat patties are illustrated in Table 3. All treatment groups showed higher antioxidant activity than control (P < 0.05) and Trolox equivalent content ranged between 0.83–5.35 mg TE/g DM for raw samples and between 0.98–4.05 mg TE/g DM for cooked samples. In treatment groups the highest antioxidant activity was observed in the treatment 20R and the lowest in 10Y. The same trend was observed in total polyphenol content. Concentration varied between 1.01–3.26 mg GAE/g DM

Table 3. Effect of onion skin water extracts on antioxidant activity (DPPH) and total polyphenol content (TPC) in raw and cooked pork patties

Type of meat patty	DPPH (mg TE/g DM)		TPC (mg GAE/g DM)	
	raw	cooked	raw	cooked
Control	0.83 ± 0.00^{eB}	0.98 ± 0.00^{eA}	1.01 ± 0.0^{eA}	0.97 ± 0.01^{dA}
10Y	1.93 ± 0.02^{dA}	1.07 ± 0.02^{dB}	1.45 ± 0.04^{dA}	1.3 ± 0.00^{cB}
10R	2.71 ± 0.00^{cA}	1.68 ± 0.02^{cB}	1.93 ± 0.00^{cA}	1.86 ± 0.07^{bA}
20Y	2.84 ± 0.00^{bA}	2.25 ± 0.0^{bB}	2.46 ± 0.08^{bA}	1.83 ± 0.02^{bB}
20R	5.35 ± 0.06^{aA}	4.05 ± 0.01^{aB}	3.26 ± 0.09^{aA}	2.97 ± 0.03^{aB}

$^{a–e}$means within a column with the same letter do not differ significantly (P > 0.05); $^{A–B}$means within a row with the same letter do not differ significantly (P > 0.05); *For abbreviations see Table 1

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for raw samples and 0.97–2.97 mg GAE/g DM for cooked samples, and all enriched groups with OSWEs had significantly ($P < 0.05$) higher polyphenol concentration compared to control. The highest polyphenol concentration was found in treatment 20R which is in agreement with AOA assay, and the lowest value was found in group 10Y.

As can be seen in Table 3, there are significant ($P < 0.05$) differences between raw and cooked patties with antioxidants in TPC and AOA. It is obvious that the heat treatment reduced antioxidant activity and also decreased the content of total polyphenols in groups with antioxidants, except for the group 10R.

Also, a strong positive linear correlation ($R^2 = 0.927$) between AOA and TPC in meat patties was found, which is illustrated Figure 3. Kim and Kim (2006) reported that DPPH radical scavenging activity is affected by the quercetin level in onion, which is the main phenolic compound in onion skin.

Lipid oxidation (TBARS values). Fat oxidation can negatively affect sensory and hygienic attributes of meat products, thus its inhibition is very important (Rohlík *et al*. 2013). The effect of different treatments on the concentration of MDA in cooked pork patties is shown in Table 4. Content of MDA at day 0 ranged from 0.11 to 0.30 μg MDA per g of sample and from 0.12 to 2.07 μg MDA/g of sample at day 5, respectively. Storage time significantly ($P < 0.05$) influenced TBARS values. For example, the control sample had an almost sevenfold increase in MDA content after 5 days. However, in group 20R, there were no significant ($P > 0.05$) differences after five days of storage. Statistically highest ($P < 0.05$) TBARS values were observed in control samples compared to all other treatments, but no significant differences ($P > 0.05$) were observed between groups with antioxidants. These results suggest that OSWEs, at a concentration of 10%, radically inhibit fat oxidation.

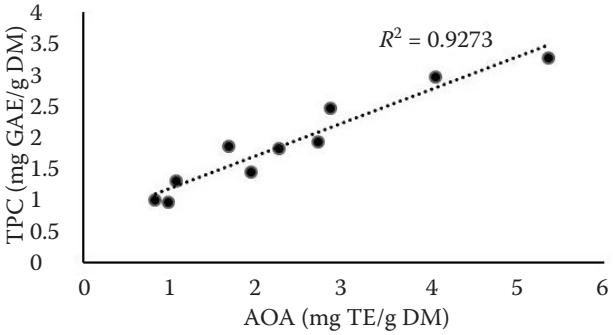

Figure 3. The relationship between total polyphenol content (TPC) and antioxidant activity (AOA) in meat patties

Table 4. Effect of onion skin water extracts on lipid peroxidation (TBARS) values in cooked pork patties under refrigerated conditions at day 0 and 5

Sample	TBARS (μg malondialdehyde/g)	
	day 0	day 5
Control	0.30 ± 0.03^{aB}	2.07 ± 0.09^{aA}
10Y	0.15 ± 0.01^{bB}	0.19 ± 0.00^{bA}
10R	0.15 ± 0.01^{bB}	0.26 ± 0.01^{bA}
20Y	0.11 ± 0.01^{dB}	0.19 ± 0.01^{bA}
20R	0.13 ± 0.01^{bA}	0.12 ± 0.01^{bA}

$^{a–b}$means within a column with the same letter do not differ significantly ($P > 0.05$); $^{A–B}$means within a row with the same letter do not differ significantly ($P > 0.05$); *for abbreviations see Table 1

Our results are in agreement with other authors who reported high effectivity of onion skin ethanol extracts in relation to meat lipid oxidation, and also that red onion skin ethanol extracts showed better results than yellow onion skin extracts (Albishi *et al*. 2013; Shim *et al*. 2012).

Sensory analysis. Sensory analysis of cooked pork patties was performed at day 0. The parameters tested were: overall acceptance, appearance, colour, flavour, texture and odour. Sensory evaluation results are illustrated in Figure 4.

Assessors detected the differences between all groups of patties, however, they were not significant ($P > 0.05$). Results indicate that the addition of OSWE at a concentration of 10 or 20% has only a small ef-

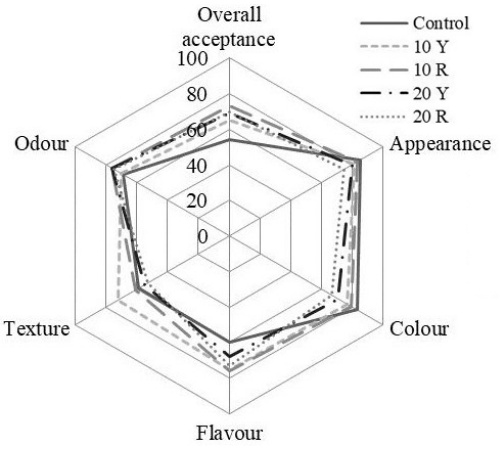

Figure 4. Sensory evaluation of meat patties with addition of antioxidants

Control – no antioxidant; 10Y–20Y – meat patty (10–20% of yellow onion skin water extract); 10R–20R – meat patty (10–20% of red onion skin water extract)

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fect on the sensory attributes of cooked meat patties. This might be influenced by trace concentrations of sulphur and alk(en)yl cysteine sulphoxides in onion skin, which are the main precursors of onion flavour (Benítez *et al*. 2011). However, our results are in disagreement with other authors who added onion skin ethanolic extracts to meat products and reported negative effects on sensory parameters (Alakahoon *et al*. 2013). Nevertheless, the authors admit that the change of the recipe can solve the problem with deterioration of sensory properties.

CONCLUSIONS

OSWEs showed positive effects on the selected properties of meat patties. They significantly increased the antioxidant activity and total polyphenol content of meat patties, at a concentration of 10%, and also provided good protection of fat against oxidation. High antioxidant activity of OSWEs is caused mainly by the presence of quercetin and quercetin monoglucoside. Our results indicate that the water extracts from onion skin, which is a waste material, can be used as a good source of antioxidants for the enhancement of cooked pork patties. There is no other data available in existing literature that can be compared with our results regarding the usage of water as an extraction agent. There are only a few references about utilization of onion skin as a source of antioxidants for meat products, but ethanol or subcritical water were used as an extraction solvent, which is, at the present time, difficult for practical application. Our simple extraction method provides a possible application for the food industry, however, more research is needed.

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