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## Agro-industrial tomato by-products and extraction of functional food ingredients

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### Abstract

Agro-industrial by-products are important sources of functional food ingredients with commercial interest to decrease the volume of by-products and also to improve the economic viability of processes, by producing added value ingredients. For this purpose, application of the supercritical carbon dioxide (SC-CO<sub>2</sub>) technology to add value to tomato (cultivar 'Admiro' F1) by-products (peel, seeds and small amount of pulp) was tested. Optimisation of extraction parameters of high value-added ingredients (oleoresin with 60% *cis*-lycopene isomers concentration) was carried out and antioxidant effect of extract in cell culture was analysed.

The extract obtained at optimised conditions (52°C temperature, a 55 MPa pressure and a 180 min extraction time), was characterized for total lycopene and lycopene different isomers (15-*cis*-lycopene, 13-*cis*-lycopene, 9-*cis*-lycopene, 7-*cis*-lycopene, *trans*-lycopene and 5-*cis*-lycopene) and the antioxidant effect of extracts was evaluated by assessing the production of the reactive oxygen species (ROS) by murine macrophage J774 cell culture.

Results showed that the SC-CO<sub>2</sub> extracts of tomato by-products (peel, seeds and small amount of pulp) is a potential source of *cis*-lycopene isomers (the total content of *cis*-lycopene isomers in the SC-CO<sub>2</sub> extract was 60%) oleoresin with promising applications in food and pharmaceutical industries.

Key words: antioxidant, lipophilic extract, lycopene, *Lycopersicon esculentum*, *trans/cis*-isomers.

### Introduction

The industrial processing of tomato (*Lycopersicon esculentum* Mill.) fruits into tomato products generates large amounts of by-products (peel, pulp and seeds). These by-products create major disposal problems for the industry in terms of costs and potential negative impact on the environment, but they also represent a promising, low-cost source of carotenoids (primarily lycopene) which may be used in the end-products because of their favourable nutritional and technological properties (Silva et al., 2014). The human diet can help reduce the risk of certain diseases and improve the quality of life (Korhonen, 2002; Kaur, Das, 2011). These concepts have led to the introduction of a new category of health-promoting foodstuffs, i.e.

functional food. Functional food is “any fresh or processed food that is claimed to have a health promoting and/or disease-preventing property beyond the basic nutritional functions of supplying nutrients” (Kaur, Das, 2011). The demand for information about functional food has increased. These foods may help prevent diseases, reduce the risk of developing a disease or enhance health (Kaur, Das, 2011). Fruits and vegetables have a wide range of various phytochemicals that may provide different health benefits (Shahidi, 2009; Viskelis et al., 2010). Scientists are trying to assess the biological activities of different phytochemicals found in fruits and vegetables to fabricate different functional foods (Bruno et al., 2007; Granato et al., 2010; Urbonavičienė et al., 2015).

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Humans are not capable of synthesizing lycopene *de novo* which implies that the amount of lycopene available for metabolic functions in the human body depends on the lycopene content in the food products consumed. It is important to highlight the fact that the absorption of lycopene from dietary sources occurs within the range of 10% to 30% in humans. Consequently, lycopene uptake is never complete and more than 70% will not be absorbed (Colle et al., 2010; Holzapfel et al., 2013). The biological activity of *cis*-lycopene isomers is different from the all-*trans*-isomers. It has been well documented that *cis*-lycopene is more bioavailable than *trans*-lycopene *in vitro* and *in vivo* probably because *cis*-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons (Fernández-García et al., 2012). Therefore, it is desirable to develop products with increased *cis*-lycopene content. The isomerization of lycopene is influenced by various technological processes and factors such as temperature, light (Urbonaviciene et al., 2017), supercritical CO<sub>2</sub> (Urbonaviciene, Viskelis, 2017).

The biochemical reactions that take place in the cells and organelles of human body support normal vital functions. Internally, free radicals are produced as a normal part of metabolism within the mitochondria, peroxisomes, inflammation processes, phagocytosis, arachidonate pathways, ischemia and physical exercise. The external factors that promote the production of free radicals, especially reactive oxygen species (ROS), are smoking, environmental pollutants, radiation, pesticides and industrial solvents (Lobo et al., 2010). In normal cellular metabolism, oxygen derivatives are neutralized or eliminated owing to the presence of a natural defence mechanism that involves enzymatic antioxidants (glutathione peroxidase, superoxide dismutase and catalase) and water or fat-soluble non-enzymatic antioxidants (biologically active compounds). The disturbed balance between production of ROS and their elimination leads to a state known as oxidative stress that induces damage to the DNA, proteins and lipids (Wiernsperger, 2003). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are the only enzymes whose primary function is to generate ROS, which are toxic to pathogens, but also may damage the surrounding normal tissues and cells, causing various abnormalities, such as infection, arteriosclerosis, neurodegenerative diseases and inflammation. Biologically active compounds of plant origin have anti-inflammatory properties, although the mechanisms of their action are not yet fully understood.

Extraction applying novel processing technologies such as supercritical fluid extraction is developing fast due to the increasing consumer demand for clean and green extraction techniques with minimal use of organic solvents. In addition, supercritical carbon dioxide (CO<sub>2</sub>) has higher selectivity for extraction of some value added ingredients from various plant materials including agro-industrial by-products (Lenucci et al., 2010; Perretti et al., 2013; Silva et al., 2014). Despite

considerable research focused on the supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction of tomato by-products, deeper knowledge concerning lycopene extracts and their biological activities is still lacking. Some studies have demonstrated the efficacy of SC-CO<sub>2</sub> for the extraction of lycopene from tomatoes. However, only the effect of few SC extraction parameters on the total yield of lycopene and carotenoids from tomatoes and tomato by-products, have been described (Lenucci et al., 2010; Perretti et al., 2013; Azabou et al., 2016). In addition, most studies focused on the amount of total lycopene extracted, whereas composition of lycopene isomers was not characterized. Different parameters of SC-CO<sub>2</sub> extraction can have individual or combined effects not only on the total extraction yield of lycopene but also on the composition of the extract obtained. Most commonly, in experiments with cells, oxidative damage induced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is measured after preincubation of cells (of various types) with carotenoids, but not with lycopene, especially with *cis*-isomers (Linnewiel-Hermoni et al., 2015; Kumar et al., 2013). Despite extensive studies on the biological activities of lycopene *cis*-isomers, detailed information about the antioxidant activities of SC-CO<sub>2</sub> *cis*-lycopene extracts is limited.

The aim of this study was to evaluate the composition of the SC-CO<sub>2</sub> *cis*-lycopene extract from tomato by-products and the antioxidant effect of extracts by assessment of the production of the reactive oxygen species (ROS) by murine macrophage J774 cell culture.

## Materials and methods

**Materials.** The experiments were carried out in 2016–2017 at the Laboratory of Biochemistry and Technology of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry and at the Neuroscience Institute, Lithuanian University of Health Science.

The following analytical grade chemicals were used: hexane (Carl Roth GmbH, Karlsruhe, Germany), acetone (Stanchem, Poland), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), dimethylsulfoxide (DMSO), Hoechst 33342, propidium iodide (PI), 2',7'-dichlorofluorescein diacetate (DCHF-DA), phorbol 12-myristate 13-acetate and arachidonic acid ("Sigma", USA), phosphate-buffered saline (PBS) ("Sigma"), horseradish peroxidase ("Sigma"), Amplex Red ("Sigma"), Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, USA). The HPLC-grade solvents, including hexane, methanol, methyl-*tert*-butyl ether, tetrahydrofuran and isopropanol were obtained from Sigma-Aldrich (Germany), methanol and methyl *tert*-butyl ether as HPLC solvents were purchased from Sigma-Aldrich (HPLC grade  $\geq 99.8\%$ ) and *trans*-lycopene (HPLC-grade, all-*trans*-lycopene, from tomato, powder) standard was from Sigma-Aldrich. Carbon dioxide (99.9%) was obtained from "Gaschema" (Jonava distr., Lithuania).

**Sample preparation.** Fully ripe red tomato (*Lycopersicon esculentum* Mill., cultivar 'Admiro' F1) was used in this study. Fruit was washed, dried and processed. The tomato juice with pulp was separated from seeds and peels. By-products (peels, vascular tissues, seeds and small amounts of pulp) of tomatoes of 'Admiro' F1 were freeze-dried using a freeze-dryer FD8512S (ilShin® Europe, The Netherlands) and were ground to a powder using a knife mill GM200 (Retsch, Germany). The powder was weighed immediately after collection and refrigerated at  $-20^{\circ}\text{C}$  prior to the extraction with SC- $\text{CO}_2$ .

**Supercritical fluid extraction with  $\text{CO}_2$ .** The supercritical carbon dioxide (SC- $\text{CO}_2$ ) experiments were carried out using supercritical fluid extractor SFT-150 (Supercritical Fluid Technologies, USA). Each extraction was performed using a 15 g sample of ground freeze-dried tomato by-products. Each sample was loaded into a 500 mL thick-walled stainless steel cylindrical extractor vessel with an inner diameter of 14 mm and a length of 320 mm. To avoid system clogging, the sample was placed between two layers of cotton wool. The temperature of the extraction vessel was controlled using a surrounding heating jacket. The volume of  $\text{CO}_2$  consumed was measured by a ball float rotameter and a digital mass flow meter in standard litres per minute ( $\text{SL min}^{-1}$ ) at standard state ( $P_{\text{CO}_2} = 100 \text{ kPa}$ ,  $T_{\text{CO}_2} = 20^{\circ}\text{C}$ ,  $\rho_{\text{CO}_2} = 0.0018 \text{ g mL}^{-1}$ ). The process consisted of static (10 min) and dynamic extraction steps. The static extraction time was included in the total extraction time.

The extracts were collected in amber glass flasks and cooled in an ice bath. The collected extracts were kept at  $-22^{\circ}\text{C}$  until analysis. Lycopene and its *cis*-isomer content in extracts from tomato by-products were determined using HPLC.

**Extraction of carotenoids.** The total lycopene content was determined according to Poojary and Passamonti (2015) with slight modification. The sample (1.0 g) was extracted repeatedly with 30 mL of acetone in *n*-hexane (1:3 v/v) for 15 min each time until the absorbance of the extract at 503 nm was lower than the instrumental noise (0.5 mAU). These extracts were combined and the total lycopene content (considered as 100% yield) was analysed using HPLC.

**High-performance liquid chromatography (HPLC) analysis of lycopene and its isomers.** The extracts and freeze-dried powder was prepared for HPLC analysis. For analysis 0.1 g of oleoresins after SC- $\text{CO}_2$  extraction was dissolved in 10 ml hexane and tetrahydrofuran with 1% butylated hydroxytoluene (BHT) solution (4:1 v/v). The HPLC system used was a liquid separation module Waters 2695 ("Waters", USA). Elution of materials was monitored by 2489 UV-Visible (UV-Vis) detector ("Waters"). Detection of lycopene was 473 nm. The lycopene *cis*-isomers for photo array PAD 2998 ("Waters") analysis detection were from 200 to 600 nm. Chromatographic separations were performed on a RP-C30 column 5  $\mu\text{m}$ , 250  $\times$  4.0 mm (YC-Europe GmbH, Germany) connected to a C30 guard column

5  $\mu\text{m}$ , 10  $\times$  4.0 mm (YC-Europe) using a flow rate of 0.65  $\text{mL min}^{-1}$ . The chosen column temperature was  $25^{\circ}\text{C}$ . The mobile phase used consisted of methanol (solvent A) and methyl-tert-butyl ether (solvent B). Samples were injected at 40% B (held 5 min), and the gradient then changed to 83% B in 50 min. Then the gradient changed to 100% B in 5 min (held 10 min) and to 40% B in 5 min (held 10 min) (Fig. 1). High-performance liquid chromatography was performed using a modified version (Urbonavičienė et al., 2015) of the different methods and systems described by Heymann et al. (2013). Levels of *cis*-lycopene isomers are given in all-*trans*-lycopene equivalents.

**Colour measurements.** The colour of extracts was measured by a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory Inc., USA) following the method of McGuire (1992), with slight modifications. The apparatus (45/0 geometry, illuminant D65, 10 observer) was calibrated with a standard tile ( $X = 81.3$ ,  $Y = 86.2$ ,  $Z = 92.7$ ). A cylindrical glass cell filled with 3 mL of sample was placed on the top of the light source (2.5 cm opening) and covered with a white plate. The inclusion of air bubbles was prevented. The recorder X, Y and Z tristimulus values were converted to CIE  $L^*$ ,  $a^*$  and  $b^*$  colour values. Regarding light reflection, the  $L^*$ ,  $a^*$  and  $b^*$  parameters (lightness, redness and yellowness indices, respectively, according to CIE  $L^*a^*b^*$  scale) were measured, the chroma ( $C = (a^{*2} + b^{*2})^{1/2}$ ) and hue angle ( $h^{\circ} = \arctan(b^*/a^*)$ ) were calculated. The colour parameters were processed with the software *Universal V.4-10*. Colour measurements were performed in triplicate.

**Cell culture.** Murine macrophage cells of J774 (Lines Service GmbH, Germany) were used for the experiments. Murine macrophage cells of J774 were maintained in DMEM + 10% fetal calf serum + penicillin (100 U  $\text{mL}^{-1}$ ) / streptomycin (100  $\mu\text{g mL}^{-1}$ ) medium at  $37^{\circ}\text{C}$  in a humidified atmosphere containing 5% of  $\text{CO}_2$ . Every 3–4 days, the cells were subcultured according to the previously described method (Raudone et al., 2014) with a slight modification.

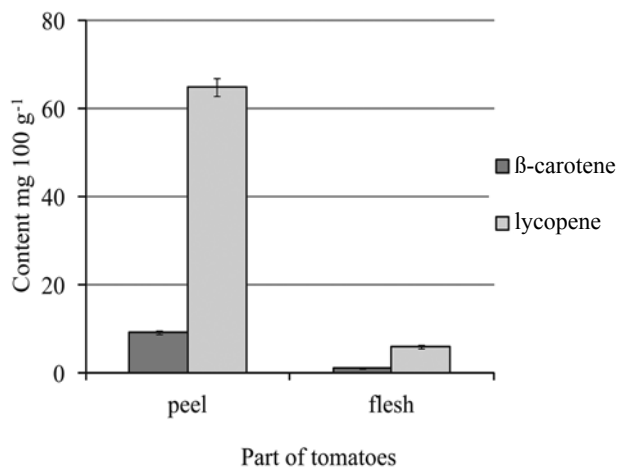
**Measurement of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) production in the macrophage culture.** In the indirect model, a murine macrophage J774 cell suspension (3  $\times$  10<sup>5</sup> cells per mL) was dispensed into 6 well plates with 1 mL medium for 2 hours (for the adherence of cells). After 2 hours, different concentrations of lycopene extract solution (0.2 and 1.2  $\mu\text{M}$ ) were added to the cell culture and incubated in a thermostat (at  $37^{\circ}\text{C}$  in a humidified atmosphere containing 5% of  $\text{CO}_2$ ). The control sample did not include lycopene extract, but was prepared under the same conditions. After 24 h of incubation, the medium with lycopene extract was removed; the cells were collected, carefully washed with a PBS buffer and centrifuged at 1000 rpm for 5 min at  $23^{\circ}\text{C}$ .  $\text{H}_2\text{O}_2$  production in the macrophage culture was measured fluorimetrically using a Fluoroskan Ascent plate reader (Thermo Fisher Scientific) at the excitation of 544 nm and an emission of 590 nm. The production of

H<sub>2</sub>O<sub>2</sub> was obtained by stimulating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase with 30 µM of arachidonic acid (ARA) and 10 µM of phorbol-12-myristate-13-acetate (PMA) in a medium containing 1 µM of Amplex Red and 10 U mL<sup>-1</sup> of horseradish peroxidase. Horseradish peroxidase uses Amplex Red as an electron donor for the reduction of H<sub>2</sub>O<sub>2</sub> to water; the reaction product resorufin is a colourful and fluorescent component. The fluorescence signal was evaluated according to the calibration curve of H<sub>2</sub>O<sub>2</sub> (nmol). A similar measurement procedure was performed for the direct evaluation of lycopene activity. The macrophage culture in measurement wells was directly affected by the concentrations (0.2, 1.2 and 7 µM) of lycopene extract, and the production of H<sub>2</sub>O<sub>2</sub> was measured fluorimetrically in a period of 15 min according to the previously described method (Raudone et al., 2014) with a slight modification. A selected concentration of lycopene corresponds to plasma-determined lycopene concentrations – 0.2–1.5 µM in accordance with various authors (Schierle et al., 1997; Sarkar et al., 2012).

**Statistical analysis.** All experiments were replicated three times and the results were expressed as the means ± standard deviations. Statistical analysis was performed using software SPSS, version 16.0 (USA). Data were analysed using one-way analysis of variance (ANOVA) followed by Duncan's test. The confidence interval was 95% ( $p < 0.05$ ).

## Results and discussion

According to the results of the study, the tomato peel contains high levels of lycopene, more than five times higher compared to the pulp and seeds (Fig. 1). Similar findings were obtained in the previous studies (Sharma, Le Maguer, 1996; Urbonaviciene et al., 2012), confirming that biorefinery of tomato by-products for the recovery of valuable functional ingredients (primary lycopene) is an important task.



**Figure 1.** Content of β-carotene and lycopene in tomato peel and flesh

In our previous study, the lycopene extraction procedure was optimized to obtain an extract (oleoresin) from by-products of tomato (cultivar 'Admiral' F1) in order to maximise the yield of concentration of lycopene *cis*-isomers (60%) having an isomeric composition valued for its biological functions (Urbonaviciene, Viskelis, 2017). The optimal extraction process parameters of *cis*-lycopene isomers are 52°C temperature, at a pressure of 55 MPa and 180 min extraction time. According to the results of the study, different technological parameters (temperature from 40°C to 60°C and the pressure of 55 MPa), influenced extract composition (Table 1). The investigated samples were as follows: No. 1 (40°C and 55 MPa), No. 2 (50°C and 55 MPa), No. 3 (60°C and 55 MPa) and No. 4 (55°C and 55 MPa). The total content of *cis*-lycopene isomer in the SC-CO<sub>2</sub> extract at optimal extraction conditions was 61.9%. The extract obtained at optimal conditions was used for assessing the production of H<sub>2</sub>O<sub>2</sub> in the murine macrophage J774 cell culture.

**Table 1.** Concentration of different *cis*-lycopene isomers in supercritical carbon dioxide (SC-CO<sub>2</sub>) extracts

Sample	Temperature °C	<i>trans</i> -	Total <i>cis</i> -	15- <i>cis</i>	13- <i>cis</i>	9- <i>cis</i>	7- <i>cis</i>	5- <i>cis</i>
No. 1	40	65.2 ± 1.94 b	34.8 ± 0.92 a	2.9 ± 0.09 a	3.7 ± 0.07 a	10.7 ± 0.29 a	6.4 ± 0.15 b	11.1 ± 0.33 b
No. 2	50	40.7 ± 0.85 a	59.3 ± 1.71 b	5.8 ± 0.17 b	8.3 ± 0.19 b	15.6 ± 0.45 b	10.5 ± 0.40 a	19.1 ± 0.53 c
No. 3	60	68.2 ± 0.16 b	31.8 ± 0.16 a	3.8 ± 0.10 a	4.4 ± 0.14 a	11.3 ± 0.37 a	5.9 ± 0.11 b	6.4 ± 0.12 a
No. 4	52	38.1 ± 0.13 a	61.9 ± 0.16 b	7.5 ± 0.19 c	8.7 ± 0.29 b	16.8 ± 0.59 b	9.3 ± 0.29 a	19.6 ± 0.43 c

*Note.* The numbers are means followed by standard deviations (n = 3); means within a column with different superscript letters are significantly different ( $p \leq 0.05$ ).

The changes in colour values (a\*, b\*, L, C and h°) of the samples are shown in Table 2. This slight variation of the total colour can be mostly associated with the different concentration of total *cis*-lycopene isomers in the extracts. According to Rodriguez-Amaya (2001), *cis*-isomerization of the double bond of the chromophore in a carotenoid molecule causes a slight loss in colour, small hypsochromic shift (usually 2 to 6 nm for mono-

*cis*), and hypochromic effect accompanied by the appearance of a *cis* peak in or near the ultraviolet region. The intensity of the *cis* band is greater as the *cis* double bond is nearer the centre of the molecule, which might explain the colour change.

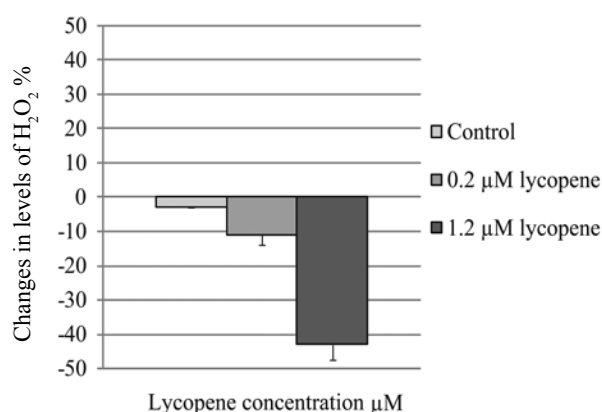
In our study the production of ROS, i.e. H<sub>2</sub>O<sub>2</sub> by macrophages was stimulated with ARA (arachidonic acid) and PMA (phorbol-12-myristate-13-acetate). The effect

**Table 2.** The changes in colour values of supercritical carbon dioxide (SC-CO<sub>2</sub>) extracts

Sample	Colour parameters				
	<i>a</i> *	<i>b</i> *	<i>L</i> *	<i>C</i>	<i>h</i> °
No. 1	4.22 ± 0.11 b	4.65 ± 0.14 b	24.55 ± 1.10 b	6.29 ± 0.19 a	47.89 ± 1.90 b
No. 2	4.95 ± 0.14 a	4.85 ± 0.09 a,b	24.18 ± 0.96 a	6.93 ± 0.21 b	43.94 ± 1.75 a
No. 3	3.74 ± 0.13 c	4.96 ± 0.11 a	24.56 ± 0.81 b	6.21 ± 0.24 a	53.02 ± 2.12 c
No. 4	5.04 ± 0.20 a	4.94 ± 0.08 a	24.23 ± 0.97 a	7.07 ± 0.20 b	44.30 ± 1.76 a

Note. *a*\* – redness, *b*\* – yellowness, *L*\* – lightness, *C* – chroma, *h*° – hue angle; the numbers are means followed by standard deviations (n = 3); means within a column with different superscript letters are significantly different (*p* ≤ 0.05).

of lycopene extract on the murine macrophage J774 cell culture was evaluated in an experimental model. PMA was chosen for the activation of ROS generation in macrophages; PMA induces protein kinase (C), which activates NADPH oxidase and triggers oxidative burst. The results demonstrate that in this model the lycopene isomer extract at the concentrations of 0.2 and 1.2 μM decreased the production of H<sub>2</sub>O<sub>2</sub> by 11% and 43%, respectively (Fig. 2).

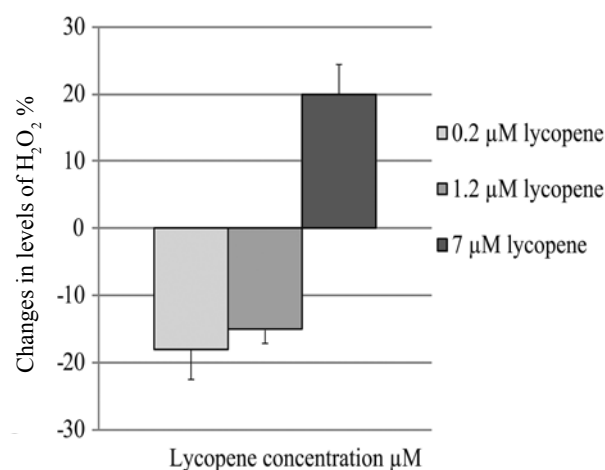


Expressed as mean ± SE (n = 3)

**Figure 2.** Indirect effect of lycopene extract on the production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a murine J774 macrophage cell culture

The measurement procedure was also performed for the direct evaluation of lycopene activity which means that the cell culture in measurement wells was directly affected by different concentrations of lycopene extract. In direct model, the extract of lycopene isomers at concentrations of 0.2 and 1.2 μM inhibit the production of H<sub>2</sub>O<sub>2</sub> by 15–18%, while concentrations exceeding the levels in plasma (7 μM) promote the concentration of H<sub>2</sub>O<sub>2</sub> by 20% (Fig. 3). In summary, the results suggest that lycopene can act on NADPH oxidase activity, affect its triggered generation of ROS and possess anti-inflammatory properties, as the ROS generated by NADPH oxidases participate in various inflammatory processes. The results indicate that the effects depend on the concentration of lycopene.

Literature suggests that *cis*-lycopene isomers have stronger *in vitro* antioxidant activity than the all-*trans* form (Müller et al., 2011). Based on literature

**Figure 3.** Direct effect of lycopene extract on the production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in murine macrophage J744 cell culture

data, the hypothesis is raised that lycopene can exert inhibition of carcinogenesis by several mechanisms: scavenging of ROS, enhancement of detoxification systems and suppression of cell cycle progression as a modulation of signal transduction pathways. Therefore, *cis*-lycopene isomers are regarded as having potentially better health benefits than *trans*-isomers. In this study, the anti-inflammatory and anti-proliferative properties of *cis*-lycopene isomers extract were examined for the first time.

Most commonly, in experiments with cells, oxidative damage induced by H<sub>2</sub>O<sub>2</sub> is measured after preincubation of cells (of various types) with carotenoids, but not with lycopene or its *cis*-isomers (Kumar et al., 2013; Linnewiel-Hermoni et al., 2015). There are several interesting studies on the effect of lycopene on protection against oxidative damage. Zini et al. (2010) preincubated human sperm samples with 2 or 5 μmol L<sup>-1</sup> lycopene before treating with 50 μmol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> and established protection against sperm DNA (measured with the comet assay) at higher concentration. According to Seo et al. (2009), lycopene protects against oxidative stress-induced cell death by preventing loss of DNA repair protein Ku70. The findings of the current research and also other studies suggest that cell culture studies might contribute useful information towards understanding the beneficial effects of carotenoids, especially their more biologically active forms, such as *cis*-lycopene isomers on human health.

In conclusion, the *in vitro* experiments demonstrated that the investigated extract of lycopene isomer (depending on the concentration of lycopene) reduces the production of H<sub>2</sub>O<sub>2</sub> in the murine macrophage J774 culture due to direct scavenging and possible effects on NADPH oxidase. The results demonstrate that the lycopene *cis*-isomer extract at the concentration of 1.2 μM decreases the production of H<sub>2</sub>O<sub>2</sub> by 43%. The results provide significant evidence for *cis*-isomer-rich lycopene extract to be considered as a dietary supplement with potential activity for the prevention of ROS damage. The knowledge regarding the anti-inflammatory properties of lycopene *cis*-isomer extract (60%) could be useful in the development of food supplements or preparations with improved beneficial effects on human health.

## Conclusions

1. The peel of tomato (cultivar 'Admiro' F1) contains about five times more lycopene than the pulp and seeds.

2. The dominant geometrical lycopene isomers in the supercritical carbon dioxide (SC-CO<sub>2</sub>) extract were 9-*cis* and 5-*cis*.

3. The total content of *cis*-lycopene isomers in the SC-CO<sub>2</sub> extract was 60% of the total lycopene content, showing that the SC-CO<sub>2</sub> extraction parameters could be modified to increase the concentration of *cis*-lycopene isomers in the extract. The use of concentrated carotenoid extracts from tomato by-products in traditional foods may improve the functional properties of the product while increasing the efficiency of the industrial processing of tomatoes.

4. The extract of isomerised lycopene (60% *cis*-isomers from the total content of lycopene) decreased generation of reactive oxygen species (ROS). The extract of isomerised lycopene decreased production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by 43% in a J774 macrophages culture. The concentrated extracts of isomerised lycopene could be applied as pharmacological agents possessing antioxidant activities.

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## Funkcionaliojo maisto ingredientų išgavimas iš pomidorų šalutinių perdirbimo produktų

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### Santrauka

Augalininkystės produktų perdirbimo šalutiniai produktai gali būti funkcionaliojo maisto sudedamųjų dalių šaltiniai, kurie yra komerciškai svarbūs, siekiant sumažinti atliekų kiekį, taip pat pagerinti procesų ekonominį efektyvumą, sukuriant pridėtinę vertę turinčius ingredientus. Šiuo tikslu buvo išbandyta ir pritaikyta ekstrakcijos superkrizinio anglies dioksidu (angl. SC-CO<sub>2</sub>) technologija, siekiant gauti didesnės pridėtinės vertės ingredientus iš veislės 'Admiral' F1 pomidorų šalutinių perdirbimo produktų (žievelės, sėklų ir nedidelio kiekio minkštimo). Buvo optimizuotas didesnės pridėtinės vertės ingredientų ekstrakcijos procesas (oleorezinas su 60 % *cis*-likopeno izomerų koncentracija) ir ištirtas antioksidacinis aktyvumas išgauto ekstrakto ląstelių kultūroje.

Optimaliomis sąlygomis (temperatūra – 52° C, slėgis – 55 MPa, ekstrakcijos laikas – 180 min) išgautas ekstraktas įvertintas nustatant sumines likopeno ir likopeno izomerų (15-*cis*-likopeno, 13-*cis*-likopeno, 9-*cis*-likopeno, 7-*cis*-likopeno, *trans*-likopeno bei 5-*cis*-likopeno) koncentracijas ir likopeno *cis*-izomerų ekstrakto antioksidacines savybes, vandenilio peroksido (H<sub>2</sub>O<sub>2</sub>) generaciją matuojant pelės makrofagų J774 ląstelių kultūroje.

Tyrimo rezultatai parodė, kad pomidorų šalutinių perdirbimo produktų (žievelių, sėklų ir nedidelio kiekio minkštimo) superkrizinės ekstrakcijos CO<sub>2</sub> oleorezinas yra potencialus *cis*-likopeno izomerų (koncentracija siekia 60 %) šaltinis, turintis plačias pritaikymo galimybes maisto ir farmacijos pramonėje.

Reikšminiai žodžiai: antioksidantas, likopenas, lipofilinis ekstraktas, *Lycopersicon esculentum*, *trans/cis*-izomerai.