KAUNAS UNIVERSITY OF TECHNOLOGY

DALIA URBONAVIČIENĖ

LYCOPENE IN TOMATOES AND TOMATO PRODUCTS: STABILITY AND ISOMERISATION DURING PROCESSING AND STORAGE

Doctoral dissertation
Technological Sciences, Chemical Engineering (05T)

2017, Kaunas

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Scientific Supervisor:

Prof. Dr. Pranas VIŠKELIS (Kaunas University of Technology, Technological Sciences, Chemical Engineering, 05T).

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

DALIA URBONAVIČIENĖ

LIKOPENAS POMIDORUOSE IR POMIDORŲ PRODUKTUOSE: JO STABILUMAS IR IZOMERIZACIJA PERDIRBIMO IR LAIKYMO METU

Daktaro disertacija
Technologijos mokslai, chemijos inžinerija (05T)

2017, Kaunas

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LIST OF ABBREVIATIONS

ANOVA – analysis of variance
APCI – atmospheric pressure chemical ionisation
ARA – arachidonic acid
ASLT – accelerated shelf life testing
AT dark – storage conditions at ambient temperature 20 ± 1°C in absence of light
AT light – storage conditions at ambient temperature 20 ± 1°C in natural light (day and night illumination was different)
β-CAR – β-carotene
BLIS – bacteriocin-like inhibitory substances
CCD – central composite design
CFU – colony-forming units
cv – cultivar
cvs. – cultivars
DMEM – Dulbecco's modified Eagle's medium
DMSO – dimethyl sulfoxide
DNA – deoxyribonucleic acid
DW – dry weight
ΔE – total colour difference
FT – storage conditions in refrigerator temperature at 1 ± 1°C in absence of light
GRAS – generally recognized as safe
h° – hue angle
HPLC – high performance liquid chromatography
L. sakei – *Lactobacillus sakei* KTU05-6
LAB – lactic acid bacteria
LYC – lycopene
MRS – de Man, Rogosa and Sharpe media
MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH – nicotinamide adenine dinucleotide phosphate
Nd – not detected
p – significance level
P. acidilactici – *Pediococcus acidilactici* KTU05-7
P. pentosaceus – *Pediococcus pentosaceus* KTU05-8
PCA – principal component analysis
PMA – phorbol-12-myristate-13-acetate
R^2 – determination coefficient
RCMP – ready-to-cook minced meat products
ROS – reactive oxygen species
RSD – relative standard deviation
RSM – response surface methodology
SC – supercritical
SC-CO_2 – supercritical extraction with carbon dioxide
SE – standard error
SF – spontaneously fermented

TApl – tomato and apple juice blend
TCar – tomato and carrot juice blend
TCor – tomato and corn juice blend
TP – tomato by-product powder
TSS – total soluble solids
TT dark – storage conditions at thermostatically controlled temperature (37 ± 1°C) in absence of light
TT UV – storage conditions at thermostatically controlled temperature (37 ± 1°C) in UV irradiation
TTA – total titratable acidity
UV – ultraviolet
Vis – visible

1. INTRODUCTION

Relevance of the research

Nowadays food is not intended to only satisfy hunger but also to prevent nutrition-related diseases and improve optimal wellness of the consumers. The advances in understanding the relationship between nutrition and health have resulted in the development and increased the demand of functional foods. Many research studies have demonstrated a significant link between the regular intake of phytochemicals (e.g. carotenoids, polyphenols, phytosterols) and the prevention of certain diseases, such as cancer, arthritis, diabetes and cardiovascular complications. Carotenoids are naturally occurring plant pigments that are responsible for the colours of different red, green, yellow and orange fruits and vegetables. While the most studied carotenoid is beta-carotene, other carotenoids, such as lycopene, are now receiving much attention due to their higher antioxidant activity and organ-specific functionality compared to beta-carotene. Carotenoids, including the red-coloured lycopene, cannot be synthesized by humans and, therefore, have to be obtained from food sources or in a form of dietary supplements.

Lycopene is narrowly distributed in foods. It is predominantly found in tomatoes and tomato products, therefore these fruits and tomato-based products contribute a significant amount of lycopene to the human diet. More than 80% of tomatoes grown worldwide are processed into products such as tomato juice, paste, puree, ketchup, sauce and salsa. Lycopene is important mainly due to its beneficial properties for human health. Lycopene exhibits antioxidant, anti-inflammatory and anticarcinogenic properties. High antioxidant activity of lycopene results from its polyene structure with 11 conjugated double bonds. In order to exert its health effects, lycopene has to be absorbed into the blood stream and reach its site of action. In the fluids and tissues of the human body, 25–70 % of lycopene is found in the form of various *cis*-isomers. It is indicated that the biological potency of *cis*-lycopene isomers is different from that of the all-*trans* form.

Based on the literature data, *cis*-lycopene isomers have a stronger *in vitro* antioxidant activity than the all-*trans* form. Therefore, *cis*-lycopene isomers are regarded as offering potentially better health benefits than the *trans*-isomers.

In fresh, red-coloured tomatoes all-*trans*-lycopene contains 94–96% of the total lycopene content. All-*trans*-lycopene is thermodynamically the most stable form. During exposure to thermoenergy, oxygen and light, lycopene can undergo isomerisation and degradation. Isomerisation converts all-*trans*-isomers to *cis*-isomers and increases the biological properties of lycopene. The opinion is that bioaccessibility and bioavailability of nutrients are more relevant for the nutritional value of foods than the nutrient concentration. However, the actual health-related benefits of lycopene strongly depend on its bioaccessibility, i.e., the fraction of lycopene that could be released from the food matrix and made available for intestinal absorption.

The determination of the degree of lycopene isomerisation and stability during processing and storage would provide a measure of the potential health benefits of tomato-based foods. It is known that the absorption of consumed lycopene reaches

only 10% (in some cases can increase up to 30%). Furthermore, lycopene absorption from fresh tomatoes is lower than from processed products (tomato paste or sauce). The technological processing (the effects of temperature, pressure, light, duration, etc.) of tomatoes may enhance the transformation of lycopene from *trans*- to *cis*-isomers. There are other factors that affect the process of lycopene absorption. For example, consuming fat with a lycopene-containing meal increases its absorption efficiency.

The industrial processing of tomatoes 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. The main problem for the extraction of tomato by-products is the preparation of tomato by-products matrix. The recovery of lycopene and other carotenoids from tomato by-products is not straightforward, as it is revealed by the low extraction yields achievable with traditional solvent extraction procedures and new extraction methods, such as supercritical carbon dioxide extraction. For this reason, additional pretreatment of the tomato by-products could be used prior to the extraction. One of the possibilities to enhance the extraction of carotenoids could be fermentation of the plant matrix. Fermentation of the plant matrix with starter cultures reduces plant cell integrity, which improves solvent penetration and lycopene dissolution. Fermentation may increase the extraction yield of lycopene and other carotenoids.

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.

Aim and tasks of the work

The aim of this work is to evaluate the effect of different processing technologies and technological parameters on lycopene in tomatoes and tomato products, its stability and possible isomerisation during processing and storage.

The following objectives are raised to achieve the aim:

1. To develop a high performance liquid chromatography method for qualitative and quantitative analysis of lycopene and lycopene *trans*- and *cis*-isomers in tomatoes, tomato products and different isolated fractions.

2. To determine the optimal conditions (temperature, pressure, extraction time) for the extraction of non-polar fraction from tomato by-products using SC-CO_2 and to test the optimised extraction parameters in order to determine the total extraction yield and concentration of *cis*-isomers in the isolated fractions.

3. To evaluate the interaction of different technological parameters on lycopene stability and isomerisation grade in different food model systems during storage.

4. To assess the effect of fermentation with lactic acid bacteria on the recovery of carotenoids from tomatoes and tomato by-products.

5. To evaluate the application possibilities of fermented tomato fraction and

lycopene-rich extract for food purposes by assessing their effect on various characteristics of ready-to-cook minced meat products and different juice blends.

6. To assess anti-inflammatory and anti-proliferative properties of the extract with isomerised lycopene.

Scientific novelty

The following scientific novelty was achieved by fulfilling the above-listed tasks:

1. An HPLC method suitable for the separation and quantitative determination of lycopene and its *trans*- and *cis*-isomers (*5-cis; 7-cis; 9-cis; 13-cis; 15-cis*) was developed and validated for the first time.

2. The SC-CO_2 extraction of tomato by-products was optimized for the isolation of non-polar fraction rich in lycopene *cis*- isomers.

3. The effect of fermentation with lactic acid bacteria on the recovery of carotenoids from tomatoes and tomato processing by-products was studied for the first time.

4. The anti-inflammatory and anti-proliferative properties of extract with isomerised lycopene were investigated for the first time.

Practical significance of the research

The data obtained in this study may be useful for understanding the combination of the technological process factors which influence the liberation and extraction of lycopene and other carotenoids from tomatoes and tomato by-products, as well as the factors which affect the stability and isomerisation of lycopene during processing and storage. The knowledge regarding lycopene extraction, stability and isomerisation might be helpful for the development and production of lycopene *cis*-isomers-rich preparations and to manufacture foods or their ingredients with enhanced levels of bioaccessible carotenoids.

The data regarding supercritical fluid extraction of lycopene could be useful for the valorisation of tomato by-products, and could serve as the basis for creating rational technology to isolate high-value biologically active components thus improving the efficiency of industrial tomato processing.

The knowledge about tomato fermentation process with lactic acid bacteria may be useful for developing new functional foods or their ingredients.

The knowledge regarding anti-inflammatory and anti-proliferative properties of isomerised lycopene extract could be useful for developing food supplements or preparations with improved beneficial effects on the human health.

The SC-CO_2 extract from tomato by-products with *cis*-isomers (60%) has found direct application in the cosmetic industry (Supplement 6). The tomato-based juice blends fortified with isomerised lycopene extract have industrial applications (Supplement 7).

Approbation of the work

The results of the research are presented in 4 scientific publications, corresponding to the main list of the Clarivate Analytical Web of Science database,

and in 2 scientific articles in journals in other international scientific information databases. A part of the results has been published by a global publisher in three book chapters (InTech publications).

The results of the research have also been presented at 8 international scientific conferences, 1 international congress and 1 symposium (14 presentations).

Structure and outline of the dissertation

The dissertation is written in English. The dissertation consists of the following sections: a list of abbreviations; an introduction; a literature review; materials and methods; results and discussion; conclusions; references; and a list of publications relevant to the subject of the dissertation. A total of 185 references were used. The final work contains 117 pages including 16 tables and 37 figures.

Statements presented for the defence

1. A combination of different technological process parameters (extraction time, temperature, pressure) could be modified to increase the concentration of the c*is*-lycopene isomers in the final product by improving product functionality.

2. The lycopene-rich preparations could be applied as pharmacological agents possessing anti-inflammatory and anti-proliferative activities.

2. LITERATURE REVIEW

2.1. Carotenoids

Carotenoids are important micronutrients and specific health benefits have been associated with the consumption of food products rich in carotenoids, such as fruits and vegetables. Carotenoids play a very important role in human health through foods, cosmetics, nutraceuticals and pharmaceuticals. Carotenoids are naturally occurring lipophilic pigments responsible for the characteristic colours of some fruits and vegetables (which account for most yellow, orange and red colours). They have received particular attention due to their health-related functions, prevention and protection against certain types of cancers, cardiovascular diseases and macular degeneration, as well as enhancing the immune function (Palmero et al. 2014a; Rao and Rao, 2007).

Carotenoids are compounds comprised of eight isoprenoid units whose order is inverted at the centre of the molecule. All carotenoids can be considered as lycopene derivatives by reactions involving: hydrogenation, dehydrogenation, cyclization, oxygen insertion, double bond migration, methyl migration, chain elongation and chain shortening.

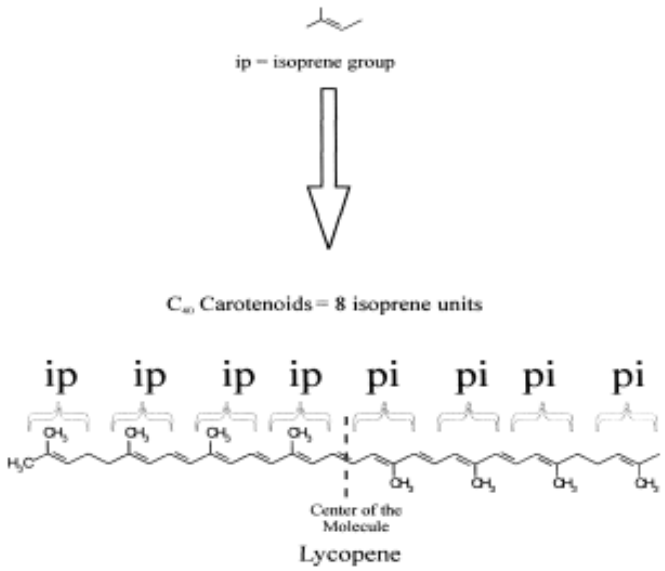


Fig. 2.1. The structure of carotenoids and the common numbering system according to the systematic nomenclature (adapted from Delgado-Vargas and Paredes-López, 2002)

Carotenoids are classified by their chemical structure as:

- carotenes that are constituted by carbon and hydrogen;
- oxycarotenoids or xanthophylls that have carbon, hydrogen and, additionally, oxygen.

Also, carotenoids have been classified as primary or secondary. Primary carotenoids group those compounds required by plants in photosynthesis (violaxanthin, β-carotene, neoxanthin), whereas secondary carotenoids are localized in fruits and flowers (α-carotene, β-cryptoxanthin, capsorubin zeaxanthin, antheraxanthin, capsanthin) (Fig. 2.2).

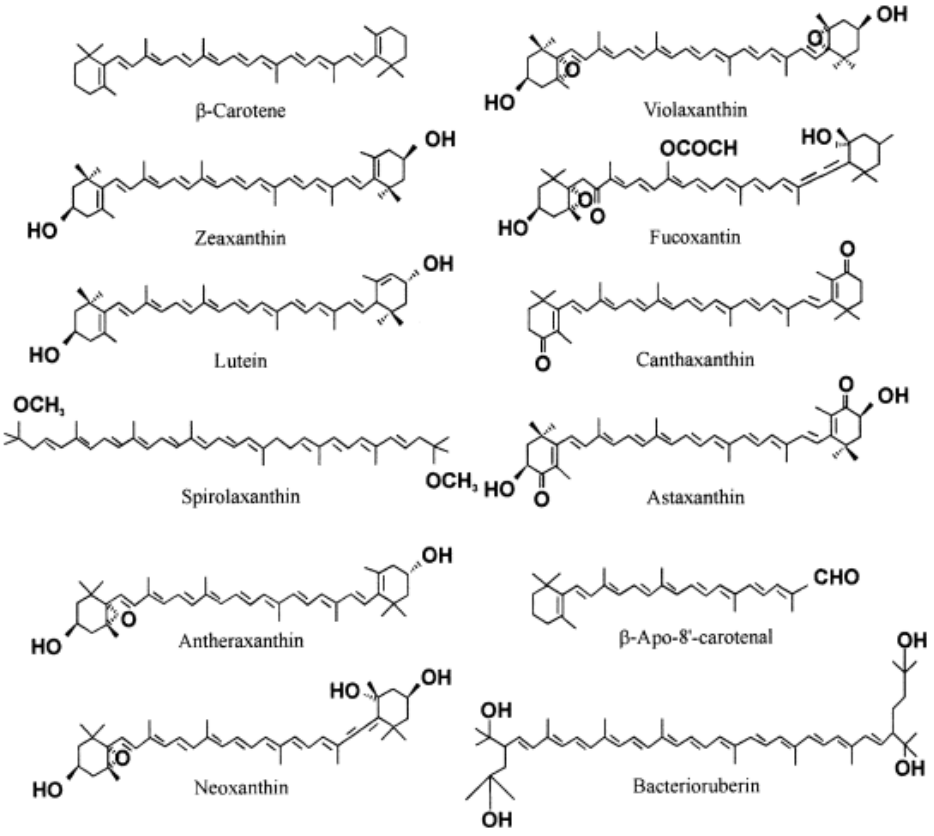


Fig. 2.2. Chemical structures of carotenoids (adapted from Delgado-Vargas, Jimenez and Paredes-López, 2000)

2.2. Lycopene molecule structure

Lycopene (ψ,ψ-carotene) is a symmetrical tetraterpene with the eight isoprene units. Lycopene has a molecular formula of $C_{40}H_{56}$, which has been attributed to its chemical structure containing 13 double bonds, 11 of which are conjugated. The structure of lycopene is the longest of all carotenoids. Due to the presence of these double bonds, lycopene can theoretically occur as 2^{11} geometrical configurations. Although most of the lycopenes are in the all-*trans*- configuration in tomatoes and other vegetables and only 71 kind of *cis*-isomers are theoretically possible because of stereo-hindrance. Figure 2.3 illustrates the structural distinctions of the predominant lycopene geometrical isomers (Lambelet et al., 2009; Zhang et al., 2012).

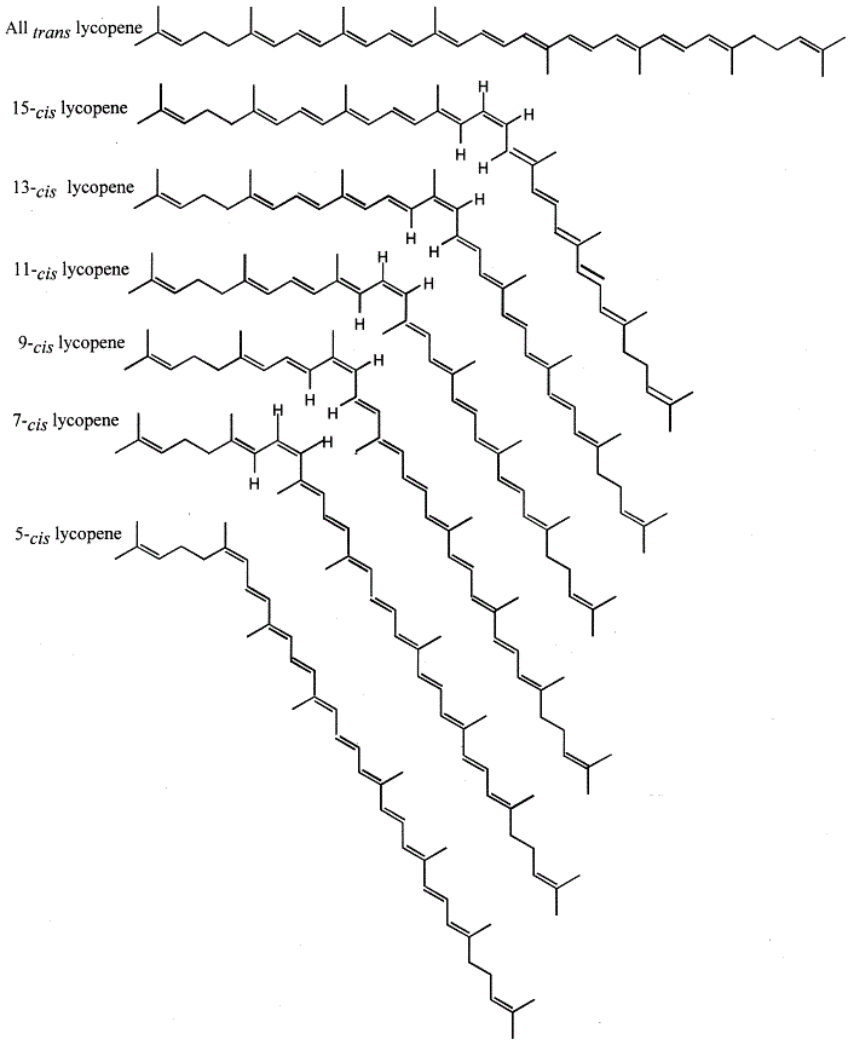


Fig. 2.3. The structure of lycopene isomers (adapted from Chasse et al., 2001)

2.3. Physical and chemical properties of lycopene

Physical and chemical properties of lycopene particularly depend on the structure of its molecule:

- molecular weight of lycopene is 536.85 Da;
- the crystal form of lycopene is long red needles from a mixture of carbon disulphide and ethanol; the powder form of lycopene is dark reddish-brown;
- each double bond reduces the energy required for electrons to transition to higher energy states, thus allowing the molecule to absorb visible light of progressively longer wavelengths, which makes it appear red. In ripe tomato fruit, lycopene takes the form of elongated, needle-like crystals that are responsible for the typical bright-red colour of ripe tomato fruit;

- lycopene exhibits the highest antioxidant activity and single oxygen quenching ability of all dietary carotenoids. The most reputed and prominent oxy-free radical species classified are hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), single oxygen ($O_2\ ^1$), organic free radicals ($RO^{\bullet}$, $ROO^{\bullet}$) and superoxide radical anion ($O_2^{\bullet -}$). Electron donor reducing compounds, such as carotenoids (including lycopene), might act as $O_2^{\bullet -}$ and $HO^{\bullet}$ scavengers, as well as O_2^1 quenchers and, therefore, could be engaged in the oxidative defence functions of plants;
- lycopene is less efficient and electron transfer is observed in both directions. The potential reduction is related to the formation of the superoxide radical anion ($O_2^{\bullet -}$) (Müller et al., 2011; Shi and Le Maguer, 2000):

$$L–C^{\bullet} + ROO^{\bullet -} \rightarrow L–C^{\bullet} + ROOH$$
$$L–C^{\bullet} + ROO^{\bullet -} \rightarrow L–ROO–C^{\bullet -}$$

- unlike β-carotene, lycopene does not have provitamin A activity because it lacks the terminal β–type ring in its structure;
- due to the acyclic structure of lycopene and its extreme hydrophobicity, it is soluble in organic solvents, such as hexane, tetrahydrofuran and chloroform (Shi, Maguer, Bryan, 2002; Strati, Oreopoulou, 2014). While most of the literature suggests that lycopene is soluble in lipids (Ax et al., 2003; Palmero et al., 2014a), the specific dissolution rates are usually not given;
- lycopene is synthesized by many plants and microorganisms but not by animals or humans;
- the conjugated double bonds of lycopene and the abundance of the large number of lycopene isomers might be created;
- lycopene undergoes two major chemical changes: isomerisation and degradation. The isomerisation and degradation have been induced by light, temperature and oxygen treatment.

2.4. The biochemical composition of tomatoes and lycopene in tomatoes

Tomato is one of the most valuable and popular vegetables worldwide. It is desirable that tomatoes are fertile and disease-resistant, and the fruit of each cultivar differs in size, shape, taste, colour, as well as firmness of skin and flesh. Tomatoes must also be resistant to transportation conditions to meet market requirements and consumer needs, as there is an increased demand for large-fruit salad-type tomato varieties. More than 80% of tomatoes grown worldwide are processed into products such as tomato juice, paste, puree, ketchup, sauce and salsa. Tomatoes and tomato-based food products, such as tomato paste, tomato sauce, tomato juice, and tomato soups are rich in carotenoid compounds.

There are many studies showing strong correlations between the intake of tomatoes or tomato lycopene and serum or plasma lycopene concentrations to the reduction and risk of cancer, coronary and cardiovascular diseases (Barba et al., 2006; Cantuti-Castelvetri, Shukitt-hale, Joseph, 2000; Ilic, Misso, 2012; Yamaguchi and Uchiyma, 2003; Yang et al., 2013). The carotenoids that have been most studied in this regard are lycopene and β-carotene. Lycopene is the predominant carotenoid found in tomatoes; in addition to lycopene, β-carotene is present in tomatoes in a

much smaller amount (Fernandez-Garcia et al., 2012) (Fig. 2.4).

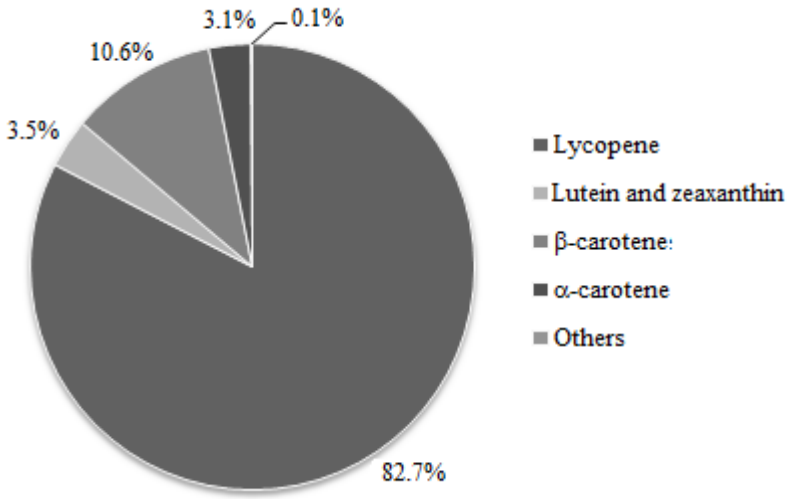


Fig.2.4. The composition of carotenoids in tomatoes (adapted from Fernandez-Garcia et al., 2012)

The characteristic red colour of a tomato is produced by a combination of carotenoid pigments, the most abundant of which is lycopene; the tomato colour is determined by the pigmentation of the skin and flesh (Brandt et al., 2006). Lycopene composes as much as 80% of the total pigmentation in tomatoes (Agarwal and Rao, 2000), and they have a consistently deep-red skin because most of lycopene is accumulated in the tomato peels (Brandt et al., 2006).

Tomato fruit are rich in organic acids, sugars, dietary fibre, pectic substances, proteins, fats, minerals (potassium, phosphorus, sulphur, magnesium, calcium, iron, copper and sodium), vitamins (B1, B2, B3, PP, C, provitamin A, I and H) and carotenoids (lycopene, β-carotene, etc.). The nutritional value, colour and flavour of tomatoes and their products depend mainly on lycopene, β-carotene, ascorbic acid and sugars, and their ratios in the fruit. The two most important carotenoids in tomato fruit are lycopene, which determines their red colour, and β-carotene, which accounts for approximately 7% of tomato carotenoids (Nguyen and Schwartz, 1999). Therefore, tomato products and their quality can be characterised by the contents of these elements. Humans get 85% of their lycopene from tomatoes and tomato products (Levy, Sharon, 2005), which is the reason why tomatoes are used in functional food products (Shi, Le Maguer, 2000; Canene-Adams et al., 2005). Epidemiological and other studies associated with the consumption of tomato products for the prevention of chronic diseases, such as cancer and cardiovascular disease, confirm that tomato products are functional foods and show that lycopene and β-carotene act as an antioxidant (Tonucci et al., 1995; Giovannucci, 1999). In order to increase the amounts of these compounds in tomato fruit, it is important to evaluate and investigate the influence of tomato genotypes on carotenoid accumulation. The literature reports that the carotenoid content in tomato fruit could

be determined by the genotypic characteristics (Abushita, Daood, Biacs, 2000; Radzevicius et al., 2009).

Currently, the food industry provides priority to change synthetic antioxidants with the "safer natural mixtures". This option has been made available through the worldwide consumer preference for natural antioxidants, some of which are added intentionally during processing and some exist inherently in foods. Between them, carotenoids (including lycopene) comprise the group of the most abundant micronutrients in fruits and vegetables (Tijskens, Evelo, 1994; Kritchevski, 1999).

The colour of flowers and fruit is caused by different types of pigments belonging to the terpenoid and phenylpropanoid classes. Carotenoids, chlorophylls and anthocyanins are the main three groups of pigments. Colour characteristics, in some plants, can be determined by domestication of agronomic traits, while in others, the increase of these pigments in tissues can occur naturally. This could be applied to the tomato (*Solanum lycopersicum* L.), which has several carotenoids, such as lycopene and β-carotene, among others. The amount of these carotenoids is principally determined by the tomato cultivar and genotype (Khachik et al., 2002). It has been established that carotene, nitrates and the amounts of sugar in fruits and root crop vegetables depend on the genotype of the plant, the meteorological conditions, fertilisation and soil composition (Johnson, 2009; Leyva et al., 2014). The levels of the essential antioxidant vitamins, in contrast with other antioxidative defences, are determined mainly by the plant's dietary supply.

One major vitamin for enriching human diets is the antioxidant vitamin C (ascorbic acid). This vitamin can counteract the oxidising effects of lipids by scavenging free radicals that have been found to be the major promoters of certain diseases. It has been demonstrated that carotenoids react cooperatively and synergistically with vitamin C, serving to regenerate a pro-oxidant radical carotenoid following the antioxidant reduction of a radical species (Kritchevski, 1999). Vitamin C, usually found in vegetables and fruits, is a natural antioxidant. Ascorbic acid plays an important role in biochemical processes, such as the formation of collagen, absorption of iron and its involvement in the immune response and the synapses. There seems to be little doubt that acids and sugar not only contribute to the sweetness and sourness of tomatoes but are also major factors in the overall flavour intensity. Since the lack of flavour is a common complaint about fresh market tomatoes, an increase in sugar and acid content could make a contribution to improve the flavour of tomatoes.

There is an opinion that carotenoids sometimes interact synergistically with other antioxidants. Mixtures of carotenoids are more effective than single compounds. A synergistic interaction of lycopene and β-carotene exists (Liu et al., 2008; Hadad, Levy, 2012).

The use of bioactive compounds in different commercial sectors, such as pharmaceutical, food and chemical industries makes it very important to extract or use the technological process that could not only increase the concentration of the bioactive compounds in the final product, but also to improve product functionality.

2.5. Lycopene isomerisation from *trans*- to *cis*-isomers

In nature, carotenoids, including lycopene, mostly exist in all-*trans*-isomers form. Thus, red tomatoes typically contain 94–96% all-*trans*-lycopene. The double bonds of the carotenoid molecules can undergo isomerization from *trans*- to mono- or poly-*cis*-isomers under the influence of heat, light, oxygen or certain chemical reactions in extracts and in tomato food products. The data in literature concludes that isomerisation from *trans*- to *cis*-isomers could be a result of the overlapping methyl group of the carbon atom adjacent to the double bond and the hydrogen. All-*trans*, *5-cis, 9-cis, 13-cis* and *15-cis* with the stability sequence are shown in Figure 2.5, which indicates that the *5-cis*-form is thermodynamically more stable than the all-*trans*-isomers (Mercadante, 2007).

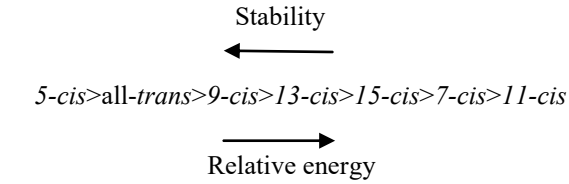
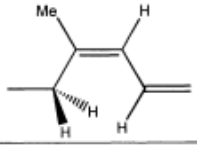
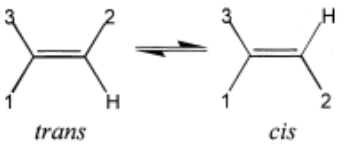
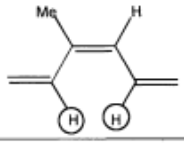
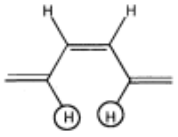
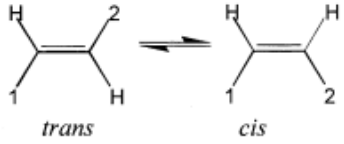
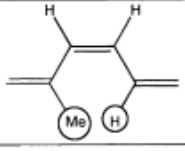


Fig.2.5. The scheme of relative energy and stability of lycopene isomers

A theoretical study on the *cis–trans* isomerisation of lycopene revealed that *5-cis*- and *9-cis*-lycopene are more stable than other isomers since their rotational barrier to re-isomerise to the all-*trans* configuration is higher ($\Delta E_r^{\ddagger}$ = 35.2 kcal/mol and 23.1 kcal/mol, respectively) than that of all other isomers ($\Delta E_r^{\ddagger}$ = 16.8 to 19.9 kcal/mol). Furthermore, the stability of *5-cis*-lycopene and *9-cis*-lycopene is also induced by their much lower relative energy compared to other isomers. In contrast, low rotational barrier ($\Delta E_r^{\ddagger}$ = 22.1 kcal/mol) and one of the highest potential energies of all mono-*cis*-isomers results in a dominant degradation of *7-cis*-lycopene during energy-rich irradiation. All-*trans*-lycopene underwent degradation while the concentration of *cis*-isomers, mainly *13-cis* and *9-cis*, increased. The investigation showed that the *5-cis*-isomer changed distinctively during lycopene storage compared to the other lycopene isomers (Chasse et al., 2001; Heymann, Raeke, Glomb, 2013).

A classification of various *cis*-lycopene isomers according to 1,4-non-bonded interactions and their extent of substitution in the carbon-carbon double bond involved are shown in Table 2.1. Not all *cis*-isomers may be of equal stability due to a number of possible 1,4 interactions. It is notable that on the basis of relative group sizes, the $–CH_3\cdots H–$ interaction (type C) appears to be the most destabilizing (Table 2.1). The order of expected stability is the following: A′>A>B>C.

Table 2.1. A classification of *cis*-lycopene isomers according to 1,4-non-bonded interactions and their extent of substitution in the carbon–carbon double bond

1,4-non bonded interaction		Isomers	Isomerisation with different degree of substitution
Type	*cis* structure		
A'		5-*cis*	
A		9-*cis* and 13-*cis*	
B		15-*cis*	
C		7-*cis* and 11-*cis*	

Since lycopene from natural plant sources exists predominantly in the all-*trans*-isomers form, it may have been assumed to be the thermodynamically most stable form in nature, for example, tomato fruits. However, it is because the biosynthesis in plants leads to the all-*trans*-form independently of its thermodynamic stability. In human plasma, lycopene is an isomeric mixture, containing at least 60% of the total lycopene as *cis*-isomers (Stahl; Sies, 1992; Ross et al., 2011).

2.6. The benefits of lycopene isomers on human health and its application to functional food

The human diet and/or its components is related to a reduction of risks related to certain diseases and even an improvement in the quality of life (Kaur, Das, 2011, Korhonen, 2002). These new opportunities have led to the introduction of a new category of health-promoting foodstuffs, i.e. functional foods. Nowadays, the development of more attractive functional food is important for the consumers' health. The relationship between diet and health has become an important factor for the consumer. The demand for information about functional food has increased. 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 function of supplying nutrients" (Kaur, Das, 2011). These foods may help prevent disease, reduce the risk of developing a disease or enhance health. More attractive ready-to-eat products are developed (Kaur, Das, 2011). Fruits and vegetables have a wide

range of benefits due to various types of phytochemicals in large amount (Shahidi, 2009). Scientists are trying to assess the health benefit of different phytochemicals in fruits and vegetables to fabricate different functional foods (Bruno, Wildman, Schwartz, 2007; Granato, 2010; Hunter et al., 2008).

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 of food products and the amount that is 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).

Dietary factors have been demonstrated to impact the absorption and distribution of carotenoids and lycopene. The bioavailability of lycopene can be affected by a number of factors, including food processing and dietary composition (Colle et al., 2010). Bioaccessibility means the fraction of the ingested lycopene "that is accessible to the body through absorption for the use in normal physiological functions and for metabolic processes" (Kopec, Schwartz, Hadley, 2010). In order to become bioaccessible, lycopene and other carotenoids must firstly be released from the food matrix and change its molecular structure from *trans* to *cis* isomers (Palmero et al., 2014b). 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 (Fernandez-Garcia et al., 2012).

The availability of lycopene from food may depend on several factors:

1) the extractability of lycopene from food may be increased by disrupting the food matrix;

2) the bioavailability of lycopene is greatly increased by *cis*-lycopene formation (Preedy, Watson, 2008, Bates, 2012);

3) the addition of lipids, such as vegetable oils, increases lycopene absorption (Shi, Le Maguer, 2000). It has been reported that lycopene is more efficiently absorbed when tomato juice is warmed with a supplemental lipid (Shi, Le Maguer, 2000);

4) β-carotene in the same dish as lycopene increases the absorption of lycopene (Bates, 2012);

A schematic representation of lycopene digestion and absorption is shown in Fig. 2.6. Firstly, lycopene must be released from the food matrix before it is incorporated into mixed micelles. "Micelles are composed of bile salts, cholesterol, fatty acids from the meal and lipophilic compounds present in the digesta" (Kopec et al., 2010). There are two ways to pass the lycopene from the micelle into the enterocyte: via passive diffusion or by a transporter known as scavenger receptor class B type I (SR-BI). Once inside the enterocyte, lycopene is packaged with other dietary lipids into chylomicrons, which are released into the lymphatic system and secreted into blood. There is little information about how lycopene and other

carotenoids in chylomicrons are accumulated by the liver and other tissues, repackaged in lipoproteins and circulated (Kopec et al., 2010; Story et al., 2010).

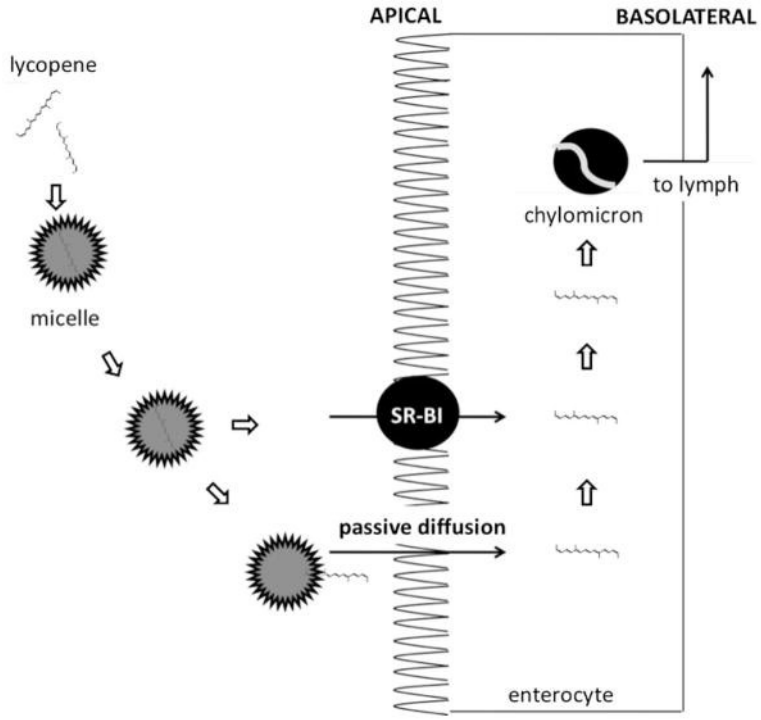


Fig. 2.6. A representation of digestion and absorption of lycopene in the small intestine (copy from Story et al., 2010)

The schematic of known and proposed dietary and genetic factors impacting the absorption and metabolism of lycopene can be seen in Figure 2.7. Multiple dietary and genetic factors have been demonstrated to impact the absorption and distribution of dietary lycopene and other carotenoids. The scheme lists factors which have been proven to be connected to lycopene absorption and distribution. Additional hypothesized factors are listed based on relationships with other carotenoids or tenuous relationships with lycopene metabolism and are denoted by Moran, Erdman and Clinton (2013) and should be the subject of future investigations.

The importance of lycopene is due mainly to its beneficial properties for human health. A review of epidemiological data indicates an inverse relationship between tomato and tomato product consumption and a risk of different types of cancer (digestive tract, cervix, breast, skin, bladder and prostate) and the occurrence of coronary heart disease (Bruno et al., 2007). The processes involved in metabolism and inflammation produce free radicals in the form of reactive oxygen species

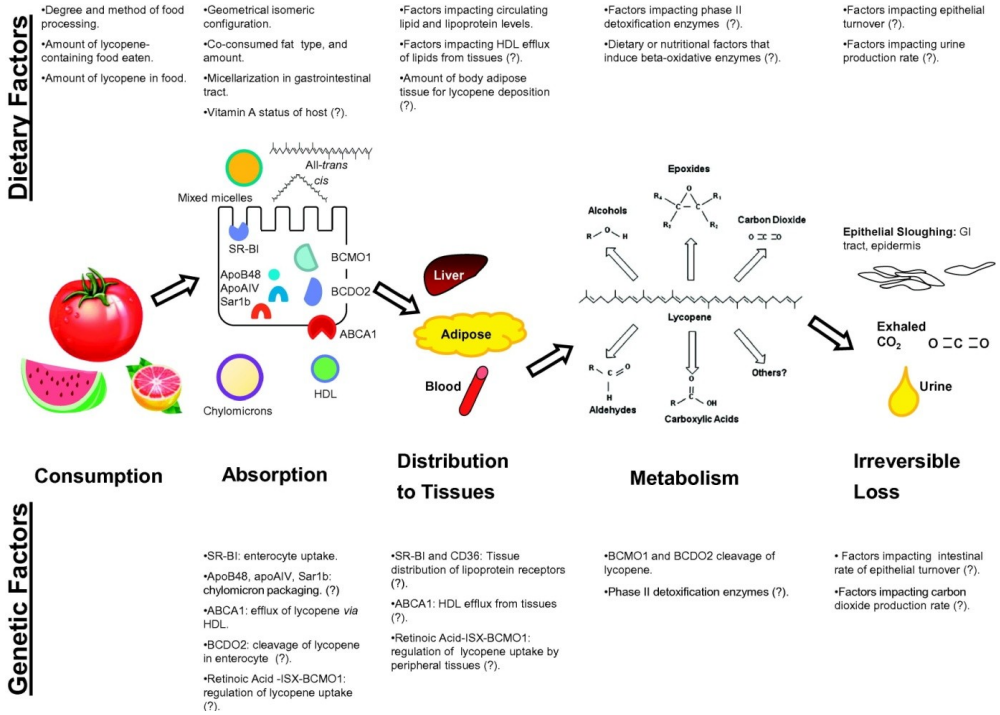


Fig. 2.7. Known and proposed dietary and genetic factors impacting the absorption and metabolism of lycopene (a copy from Moran et al., 2013)

(ROS) and reactive nitric-oxide species (RNOS). These reactive intermediates can modify and damage lipids, proteins and DNA resulting in the disruption of key cellular processes such as DNA repair, cell-cycle checkpoints and apoptosis. "Antioxidants such as lycopene can serve to absorb or scavenge the excitation energy (indicated by red asterisk) from free radicals where the excess energy is dissipated though rotational and vibrational interactions between lycopene and solvent, thereby protecting crucial cellular molecules from damage" (Nelson, Montgomery, 2003). The importance of lycopene in the maintenance of health and protection from certain diseases, such as hypertension, osteoporosis, neurodegenerative diseases, male infertility and even transmission of acquired immunodeficiency syndrome from mothers to babies (Fig. 2.8) (Nelson, Montgomery, 2003).

Moran et al. (2013) highlight the studies of lycopene concentration in human tissues. The majority of the lycopene concentration of the body was found in the adipose tissue (60–72% in males and females). The lowest concentration was found in the serum or plasma, only about 5% (in males and females) (Moran et al., 2013).

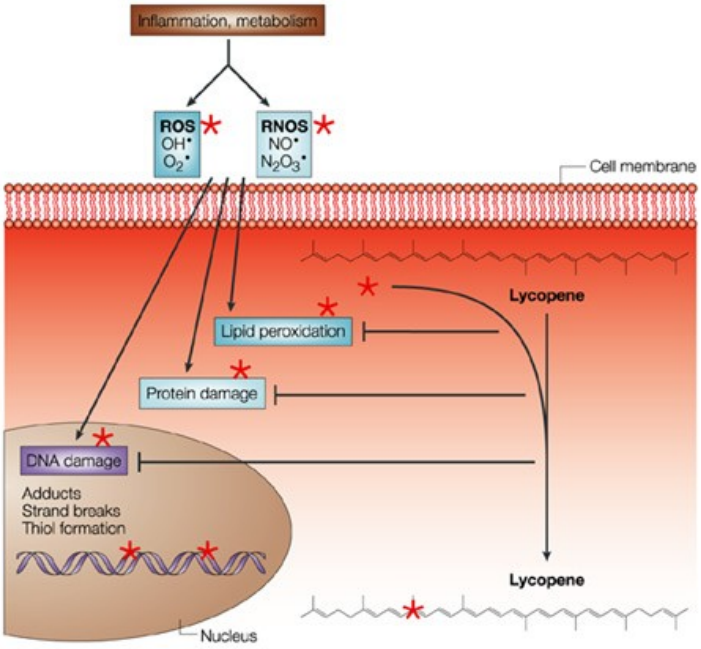


Fig. 2.8. Lycopene antioxidant activity (adapted from Nelson, Montgomery, 2003)

The publication of Mazeikiene and colleagues (2015) studied the mean lycopene intake and the main dietary sources in the ethnic Lithuanian population. The main differences in the intake of lycopene depending on age, gender and ethnolinguistic group were evaluated. The relationship between the lycopene intake and biochemical risk factors of atherosclerosis was studied. The study concluded that the mean of lycopene intake in the general Lithuanian population was one of the lowest compared to intakes reported in other European countries (Table. 2.2) (Mazeikiene et al., 2016). Despite numerous studies about the positive effect of lycopene to human health, there is no recommended daily intake. The Acceptable Daily Intake level for lycopene approved by the European Food Safety Authority is 0.5 g/kg of body weight per day. The Lithuanian scientific results showed that the mean lycopene intake was 0.7 mg/kg of body weight, which is more than 7 times lower than the acceptable daily intake.

Table. 2.2. Lycopene intake

Country	n	Lycopene intake	
		Median	Interquartile range (Q1–Q3)
France[1]	76	4.75	(2.14–8.31)
Spain[1]	70	1.64	(0.50–2.64)
United Kingdom	71	5.01	(3.2–7.28)
Ireland[1]	76	4.43	(2.73–7.13)
Holland[1]	75	4.86	(2.79–7.53)
Lithuania (2008)[2]	48	1.98	(1.16–4.06)
Lithuania	497	1.37	(0.00–4.62)

[1] O'Neill et al., 2001; [2] Mazeikiene et al., 2008

It is very important to understand how the concentration, structure and ratios of specific carotenoids (especially lycopene) affect their activity and uptake in the human body.

2.7. The bioavailability of lycopene from food: technological, analytical and nutritional implications

Nutrient bioaccessibility, defined as the fraction of an ingested nutrient released from the matrix and available for intestinal absorption, is a prerequisite for its bioavailability (Holst, Williamson, 2008) and depends on the nutrient localization in the food matrix. Lycopene is localized in the tomato chromoplasts where it appears as carotenoid–protein complexes or as solid microcrystals (Shi, Le Maguer, 2000). The food matrix in which lycopene is incorporated and which can be altered by different food processing technologies greatly influences the bioaccessibility of lycopene (Castenmiller, West, 1998). Recently, the relationship between food microstructure and nutritional food properties has attracted a lot of attention (Ellis et al., 2004; Parada, Aguilera, 2007; Lemmens et al., 2009). In this relationship, the intactness and the pectin properties (Lemmens et al., 2009) of the plant cell walls seem to play an important role. Moreover, the results achieved by Lemmens et al. (2009) showed that the microstructural properties of carrot tissue, as they affect hardness, are inversely related to β-carotene in vitro bioaccessibility. The molecular structure of a nutrient is the smallest structural level relevant for its biological role and activity (for example, *cis*- or *trans*-conformation, the number of carotenoids).

2.7.1. Extraction possibilities and solubility of lycopene

Lycopene is hydrophobic and can be dissolved in non-polar organic solvents, such as tetrahydrofuran, hexane, chloroform, acetone, but are essentially insoluble in polar solvents, such as water and ethanol. Lycopene also is sensitive to heat, oxygen, light and acids. The need for safer methods of obtaining lycopene from natural plant materials has led to supercritical fluid (SC) extraction (Zuknik, Norulaini, Omar, 2012). SC extraction is an alternative technique to conventional extraction of bio-compounds, such as lycopene, without organic solvents and the extracts can be successfully explored in food and pharmaceutical applications.

Supercritical fluids (SC) have liquid-like densities and this property gives mass transfer characteristics compared with organic solvents. The physical properties of supercritical fluids are described to be between a liquid and a gas. The advantage of using supercritical fluids is the possibility to extract specific and very sensitive compounds in changing conditions of the technological process. In order to reach the supercritical state, the substance should reach the state when pressure and temperature are beyond their respectively critical value. There is particular benefit using CO_2 in SC extraction because this supercritical solvent is:

- environmentally safe,
- non-flammable,
- non-toxic for live organisms,
- non-explosive,
- exhibits high diffusivity and high selectivity as a result of low viscosity,
- has liquid-like density,
- is readily available and inexpensive,
- is easily removed from the final product.

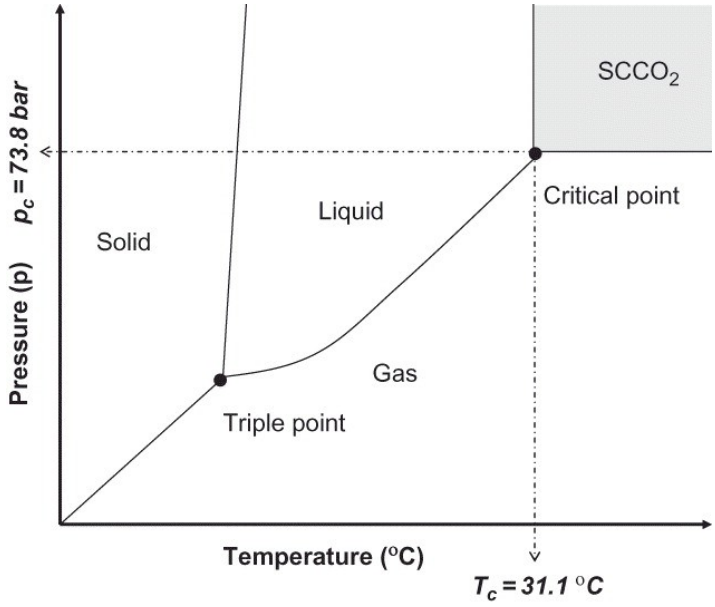


Fig. 2.9. The phase diagram of carbon dioxide (adapted from Rawson et al., 2012)

According to Zhao and Zhang (2013), the SC extraction process normally contains four steps shown in Fig. 2.10: (1) the diffusion of the supercritical fluid into the matrix; (2) the dissolution of the extracted substance into the supercritical fluid; (3) the intraparticle diffusion of the substance and the supercritical fluid into the outside of the solid surface; and (4) the flow-away of the supercritical fluid and the substance mixture from the matrix.

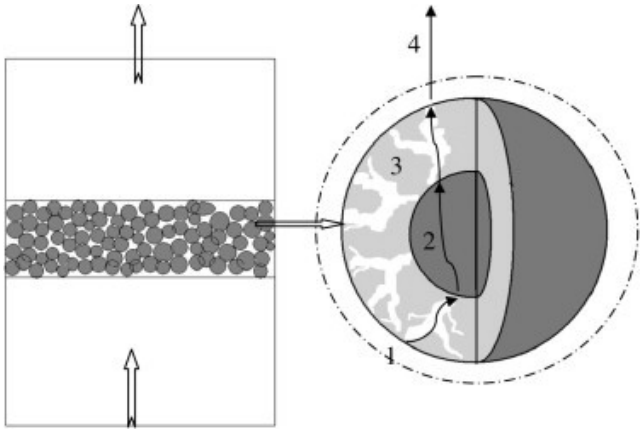


Fig. 2.10. A diagram of the SC extraction process: 1) the diffusion of the SF into the substrate matrix; 2) the dissolution of extracted substance into the SC; 3) the diffusion of the SC to the outer surface of the substrate; 4) the flow-away of the SC and the substrate mixture from the matrix (adapted from Zhao, Zhang, 2013)

The idea of using lycopene-rich by-products from tomato processing industries to be incorporated into functional food is being exercised. Fortification with lycopene in dry fermented sausage by adding dried tomato peel to meat, the enrichment of low quality edible oils by lycopene from tomato peels or tomato puree to induce thermal stability, feeding hen lycopene-rich by-products to enrich the egg yolk with lycopene, the encapsulation of supercritical fluid extract of lycopene-rich tomato pulp waste, the enrichment of extruded snacks using barley-tomato pomace blends, and mixture of lycopene and soy protein, are some of the ventures attempted (Kong et al., 2010; Kaur and Das, 2011). Liu et al. (2008) suggested that the antioxidant property of the combination of lycopene, vitamin E, vitamin C, and β-carotene mixtures was substantially superior to the sum of the individual antioxidant effects.

Food processing may improve the bioavailability of lycopene by breaking down cell walls, which weakens the bonding forces between lycopene and the tissue matrix, thus making it more accessible. Shi and Le Maguer (2000) confirm that the matrix may contribute to the stability of all *trans*-forms of lycopene in tomatoes, thus preventing the isomerisation. During food processing, the food matrix could be disrupted and lycopene may be incorporated into micelles prior to absorption. It is possible that once this disruption occurs, further isomerization of *trans*-lycopene may occur (Omoni, Aluko, 2005). The tomato matrix consists of dehydrated clumps of berry mesocarp parenchyma cells. Lycopene is present as red crystals within the chromoplasts in tomato cells. Lycopene crystals are enclosed into newly synthesised membranes originating by introflections of the inner membrane plastid envelope (Simkin, Zhu, Kuntz, Sandmann, 2003).

Thus, to solubilise and extract lycopene with organic solvents or supercritical fluids, firstly the solvent must reach these organelles penetrating through the pecto-cellulosic primary cell-wall and the membrane bilayers of cell which represent potential obstacles to the free diffusion of the fluid. The main reasons why it is

difficult to extract lycopene from the tomato matrix are the compactness of the plant tissue, which hinders solvent penetration, its transport to chromoplasts containing lycopene and the possible degradation of the carotenoid during recovery. Thus preparation of tomato matrix is necessary before the extraction procedure.

There are some studies analysing alternative extraction methods which include supercritical fluid extraction with primary enzyme-assisted preparation of the sample for the recovery of carotenoids from tomato and tomato by-products (Zuorro et al., 2011; Ranveer et al., 2013). There is evidence that fermentation may enhance the recovery of different biologically active substances from plant materials (Puri et al., 2013).

2.7.2. The use of lactic acid bacteria in the fermentation technology of fruits and vegetables

Lactic acid fermentation of vegetable products applied as a preservation method is considered as an important technology and is further investigated because of the growing amount of raw materials processed in this way in the food industry. Fermentation, defined as the extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds, is an approach to the problem of food preservation and has gained increasing attention in recent years. Consequently, certain lactic acid bacteria (LAB) with demonstrated antimicrobial properties are derived from the production of one or more antimicrobial active metabolites, such as organic acids (lactic and acetic), hydrogen peroxide, antimicrobial peptides (bacteriocins) and hydrolitical enzymes (Cleveland et al., 2001).

The relationship between food and health has been investigated for many years, therefore, the development of foods that promote health and well-being is a key research priority of the food industry (Klaenhammer, Kullen, 1999). Vegetables are strongly recommended in the human diet because they are rich in antioxidants, vitamins, dietary fibres, and minerals. The majority of vegetables consumed in the human diet are fresh, minimally processed, pasteurized, or cooked by boiling in water or microwaving, vegetables can also be canned, dried, juiced, made into pastas, salads, sauces and soups. Fresh vegetables or those that have been minimally processed have a particularly short shelf-life because they are subjected to rapid microbial spoilage. In addition, the abovementioned cooking processes can cause a number of potentially undesirable changes in their physical characteristics and chemical composition (Zia-ur-Rehman, Islam, Shal, 2003; Zhang, Hamauzu, 2004). The use of natural antimicrobial preservatives is considered to be the simplest and most valuable biological technique to keep and/or enhance the safety, nutrition, palatability and shelf-life of fruits and vegetables (Devlieghere, Vermeiren and Debevere, 2004).

Lactic acid fermentation of vegetables, currently used as a bio-preservation method for the manufacture of finished and half-finished foods, is an important biotechnology for maintaining and/or improving the safety, nutritional, sensory, and shelf-life properties of vegetables. Three technological options are usually considered for lactic acid fermentation of vegetables: spontaneous fermentation by

autochthonous lactic acid bacteria, fermentation by starter cultures that are added into raw vegetables, and fermentation of mild heat-treated vegetables by starter cultures (Hammes, Tichaczek, 1994). For thousands of years, microorganisms have been used to produce and preserve foods through the process of fermentation. Fermented foods have been adopted in various ways depending on the properties of the available raw materials and the desired features of the final products (Liu, Han, Zhou, 2011; Juodeikiene et al., 2011; Sanchez et al., 2012). Food produced by traditional methods has become popular among consumers who know that their food is manufactured from high quality raw materials, without preservatives and other synthetic additives that are characterised by unique flavour values (Clark, 2004).

Fermentation from a biochemical point of view

Bourdichon et al. (2012) describe the fermentation process as "a metabolic process of deriving energy from organic compounds without the involvement of an exogenous oxidizing agent". Fermented foods are subjected to the actions of microorganisms or enzymes. Fermentation plays different roles in food processing, such that desirable biochemical changes have occurred (Caplice, Fizgerald, 1999). The fermentation process is very important for the improvement of technological properties of preservation, such as the relative cost-effectiveness and low energy requirements, which are essential for ensuring the shelf-life and microbiological safety of the product (Liu et al., 2011). The major roles of fermentation are considered to be the following (Fig. 2.11):

1) preservation of food: the formation of inhibitory metabolites, such as organic acid (lactic acid, acetic acid, formic acid, propionic acid), ethanol, bacteriocins, etc., often in combination with a decrease in water activity (by drying or the use of salt) (Gaggia et al., 2011; Ross, Morgan, Hill, 2002);

2) improving food safety through the inhibition of pathogens (Adams, Nicolaides, 1997) or the removal of toxic compounds (Hammes and Tichaczek, 1994);

3) improving nutritional value: biological enrichment of food substrates with proteins, essential amino acids, essential fatty acids and vitamins (van Boekel et al., 2010; Poutanen, Flander, Katina, 2009);

4) organoleptic food quality: enrichment of the diet through the development of a diversity of flavours, aromas, and textures in food substrates (Marilley, Casey, 2004; Lacroix et al., 2010; Sicard, Legras, 2011; Smit, Smit, Engels, 2005);

5) decrease in cooking times and fuel requirements (Steinkraus, 1996). The interest in biopreservation of food has created a demand for more natural and minimally processed food, with particular interest in naturally produced antimicrobial agents (Cleveland et al., 2001).

Lactic acid bacteria in food fermentation and new natural antimicrobial compounds

LAB have traditionally been associated with food fermentation. LAB are generally considered beneficial microorganisms, with some strains even considered to promote good health (probiotic), and their extensive historical use contributes to

their acceptance as being GRAS for human consumption (Silva et al., 2002). LAB are used as natural or selected starters in food fermentation and exert health benefits through the antimicrobial effect produced from different metabolic processes (lactose metabolism, proteolytic enzymes, citrate uptake, bacteriophage resistance, bacteriocin production, polysaccharide biosynthesis, metalion resistance and antibiotic resistance) (Corsetti, Settanni, Van Sinderen, 2004; Zotta, Parente, Ricciardi, 2009). Spontaneous fermentation typically results from the competitive activity of a variety of autochthonous and contaminating microorganisms, which may lead to a high risk for failure. Both from the hygiene and safety perspectives, the use of starter cultures is recommended, as it leads to rapid inhibition of spoilage and pathogenic bacteria while yielding processed fruit with consistent sensory and nutritional quality (Saez-Rodriguez et al., 2009).

The interest in biopreservation of food has prompted the quest for novel antimicrobial compounds from different natural origins. The LAB of genera, such as *lactobacilli* and *lactococcus* are amongst the most important known members that have probiotic activity. LAB produce antimicrobial peptides, most frequently referred to as bacteriocins (Vaughau et al., 2001; Altuntas, Cosansu, Ayhan, 2010). Bacteriocins ensure the stability of fermented plant products, reduce microbial contamination during fermentation, inhibit the growth of moulds and delay microbiological spoilage of baked goods (Juodeikiene et al., 2009).

LAB have strong inhibitory effects on the growth and toxin production of other bacteria. This activity can occur due to the following factors: competition for available nutrients; a decrease in redox potential; the production of lactic acid and acetic acid and the resulting decrease in pH; the production of other inhibitory primary metabolites, such as hydrogen peroxide, carbon dioxide, or diacetyl; hydrolytical enzymes and the production of special antimicrobial compounds, such as bacteriocins and antibiotics. Each of these properties, particularly when combined, can be used to extend the shelf-life and safety of food products (Kalantzopoulos, 1997). Amongst the various technologies, lactic acid fermentation may be thought of as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of fruits and vegetables (Buckenhüskes, 1997). Overall, LAB are a small part (2–4 $\log_{10}$ CFU/g) of the autochthonous microbiota of raw vegetables, and their cell density is mainly influenced by the vegetable species, temperature and harvesting conditions (Buckenhüskes, 1997). The interest in the use of LAB fermentation of vegetable products stems largely from the nutritional, physiological, and hygienic aspects of the process and their corresponding implementation and production costs. LAB fermentation represents the easiest and most suitable way to increase the daily consumption of nearly fresh fruits and vegetables.

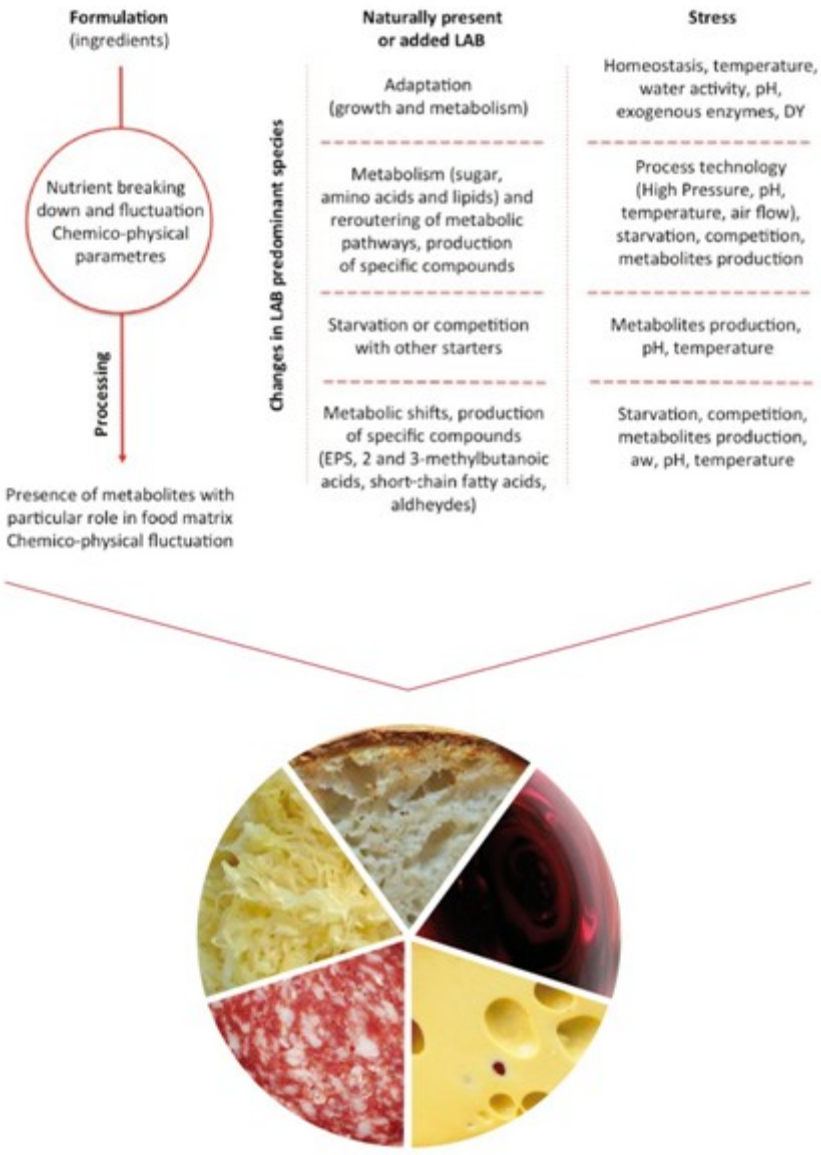


Fig. 2.11. Fermented food model: reciprocal influences between environmental fluctuation and lactic acid bacteria fermentation (Serrazanetti et al., 2013)

Unique features of fermented fruits and vegetables

Buckenhüskes and colleagues (Buckenhüskes, 1993) generally agree that fermented plant products are the "food of the future". The following factors support this idea: products can be marked as "natural" or "biological"; desirable flavour compounds are enhanced while negative flavour compounds (for example, glucosinolates) are destroyed; handling and storage (without cooling) is simple; easy methods exist for the pre-handling of raw material before further processing; desired metabolites (lactic acid, amino acids) are enriched; and the process results in the detoxification of pathogens (Maki, 2004). In Figure 2.11, the steps that mainly interest food fermentation are reported (Serrazanetti et al., 2013). Fruits and vegetables preserved using LAB with antimicrobial properties are perceived as suitable products for the human diet.

Dieticians and physicians recommend fermented fruits and vegetables due to the health-promoting properties of these foods. Fermented fruits and vegetables are low-calorie foods because they contain considerably lower quantities of sugars compared to their raw counterparts. Fermented vegetables are a source of dietary fibre, which impedes the assimilation of fats and regulates peristalsis in the intestines; they are also a valuable source of vitamin C, B group vitamins, phenolic, and many other nutrients present in the raw material. Lactic acid may also lower the gut pH, thereby inhibiting the development of putrefactive bacteria (Howarth, Wang, 2013).

Many types of fermented fruit and vegetable products exist in the world: sauerkraut, cucumber pickles and olives in the Western world; Egyptian pickled vegetables in the Middle East Indian pickled vegetables, Korean kim-chi, Thai pak-sian-don, Chinese hum-choy, Malaysian pickled vegetables and Malaysian tempoyak. Lactic acid-fermented cereals and tubers (cassava) include Mexican pozol, Ghanaian kenkey, Nigerian gari; boiled rice/raw shrimp/raw fish mixtures, such as Philippine balao-balao and burong dalag; lactic-fermented/leavened breads, such as sourdough breads in the Western world; Indian idli, dhokla, khaman and Sri Lankan hoppers; Ethiopian enjera, Sudanese kisra and Philippine puto; and Chinese sufu/tofu-ru (Peres, 2012).

Commercial distribution of these fermented products lags far behind that of fermented meat and dairy products due to a lack of standardised manufacturing protocols; in addition, their ingredients are subject to limiting and unpredictable weather and geographic conditions (Cetin, 2011). The lactic acid fermentation of vegetables currently has industrial significance only for cucumbers, cabbages and olives (Montet, Loisea and Kakhia-Rozis, 2006). Several other varieties of vegetables cultivated mainly in Southern Italy or, more generally, in the Mediterranean area, such as carrots, French beans, marrows, artichokes, capers and eggplants, may benefit from increased safety, nutritional, sensory and shelf-life properties through standardized industrial lactic acid fermentation (Di Cagno et al., 2009).

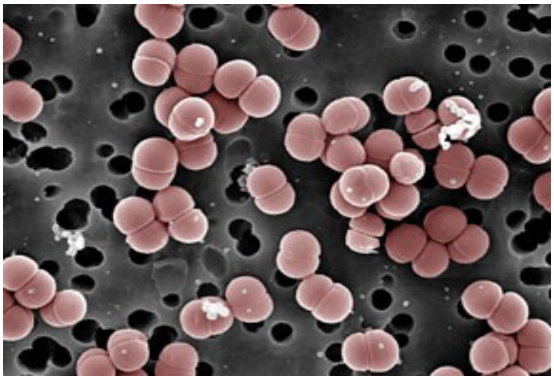


Fig. 2.12. *Pediococus pentosaceus* MI807

There is an increasing consumer demand for high quality meat products that taste good and are both nutritious and easy to prepare. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage microorganisms and common food-borne pathogens. It is therefore essential to apply adequate preservation techniques to maintain its safety and quality (Aymerich, Picouet, Monfort, 2008). The processes used in meat preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation seek to minimise additional deteriorative changes in colour and oxidation (Zhou, Xu, Liu, 2010). The most investigated new preservation technologies for fresh meat involve non-thermal inactivation, such as high hydrostatic pressure, novel packaging systems, including modified atmosphere packaging and active packaging, natural antimicrobial compounds and biopreservation. Storage life is extended and safety is increased by using natural or controlled microflora, including the extensively studied LAB and their antimicrobial products, such as lactic acid and bacteriocins (Fig. 2.12 and 2.13).

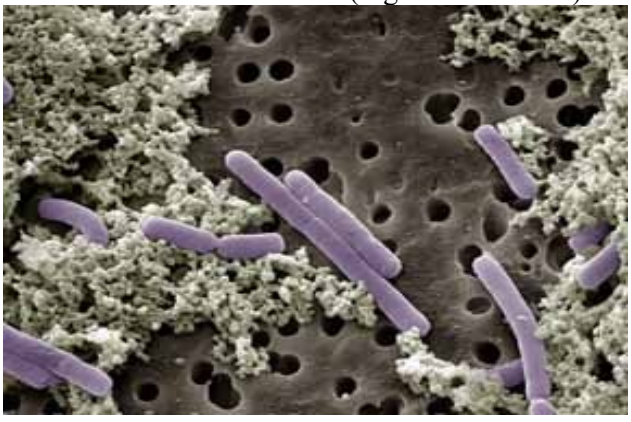


Fig. 2.13. *Lactobaccilus sakei*

Bacteriocins are a heterogeneous group of antibacterial proteins that vary in their spectrum of activity, mode of action, molecular weight, genetic origin and

biochemical properties (Stiles, Hastings, 1991). The destruction of the total BLIS (bacteriocin-like inhibitory substances) activity after treatment with proteinase K, trypsin, pepsin and chymotrypsin indicates that antimicrobial substances produced by the tested LAB possess a proteinaceous nature.

They might be bacteriocins because protease sensitivity is a key criterion in the classification of antimicrobial substances as BLIS (Klaenhammer, 1993). It was proposed that due to their broad inhibition spectrum, the presence of BLIS and organic acids in the tested LAB is an indication that these bacteria can be used widely in the food industry as bio-preservatives.

2.8. Concluding remarks

Based on literature review, there is a number of research papers describing different determination methods of lycopene. However, among all the research papers analyzed we could not find a universal method, sufficiently reliable and sensitive for the HPLC determination of lycopene and all its separate isomers (from *5-cis-* to *15-cis-*). To the best of our knowledge, there is no precise description of such HPLC method and validation and determination of the different lycopene *cis-*isomers (*5-cis-, 7-cis-, 9-cis-, 13-cis-, 15-cis-*) in different food materials (fresh tomatoes, tomato-based food products, model food systems, fermented tomato fractions and products).

Despite considerable research focused on the extraction of tomato by-products, deeper knowledge concerning lycopene isomerization and its optimized extraction without the use of organic solvents but with the application of mathematical models is still lacking. To the best of our knowledge, the response surface methodology was not yet applied for the optimization of SC-CO_2 parameters in order to investigate their influence on the yield of *cis*-lycopene isomers. Some studies have demonstrated the efficacy of SC-CO_2 for the extraction of lycopene from tomatoes. However, only the effect of some 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, Troilo, Bravi, Marconi, Galgano, Fantozzi, 2013; Azabou, Abid, Sebii, Felfoul, Gargouri, Attia, 2016). In addition, most studies focused on the amount of total lycopene extracted, whereas lycopene isomer composition and their extraction efficiency were not characterized. However, different parameters of SC 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.

A major problem in the analysis of food carotenoids, including lycopene, is the sample preparation and extraction. The recovery of lycopene and other carotenoids from tomato by-products is not straightforward, which is obvious from the low extraction yields achievable with traditional (solvent) extraction procedures. For this reason, additional pretreatment of tomato by-products prior to their extraction can be beneficial. It must be taken into account that the liberation of bioactive compounds, such as carotenoids, from the plant matrix can be influenced by their physical form

of deposition in plant chromoplasts (Schweiggert et al., 2012). Recent scientific studies suggested a novel, enzyme-assisted procedure for the deconstruction of primary cell-wall of tomato parenchyma cells as a pretreatment method for an improved SC-CO_2 extraction of lycopene (Zuorro et al. (2011)). To the best of our knowledge, there is no data available about the effect of LAB fermentation on carotenoids present in tomatoes and their possible changes during the process.

There is a number of factors which influence the stability of biologically active constituents in tomatoes and tomato products. Although there are some data concerning the stability of carotenoids during tomato processing (George et al., 2011; Vallverdú-Queralt et al. 2015), there is still a lack of knowledge about the combined effect of different technological factors on the stability of individual carotenoid isomers in foods during long-term storage.

3. MATERIALS AND METHODS

3.1. Reagents and standards

The following analytical grade chemicals were commercially available: hexane (Carl Roth GmbH, Karlsruhe, Germany), acetone (Stanchem, Poland), 2,6-dichlorophenolindophenol sodium salt were from Merck (Darmstadt, Germany), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), dimethylsulfoxide (DMSO), Hoechst 33342, propidium iodide (PI), 2′,7′-dichlorofluorescin diacetate (DCHF-DA), phorbol 12-myristate 13-acetate (Sigma, USA), phosphate-buffered saline (PBS) (Sigma, USA), horseradish peroxidase (Sigma, USA), Amplex Red (Sigma, USA), 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).

The *trans*-lycopene (HPLC-grade, *all-trans* lycopene, from tomato, powder) and β-carotene (HPLC-grade, synthetic, ≥93%, powder) standards were used (Sigma-Aldrich, Germany).

Carbon dioxide (99.9%) was obtained from Gaschema (Jonava distr., Lithuania).

3.2. 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.

3.3. Qualitative and quantitative analysis and validation of lycopene isomers and β-carotene using high-performance liquid chromatography

The HPLC system used in this research was the Waters 2695 liquid separation module (Water Corporation, USA.). The elution of materials was monitored by UV-Visible detector (UV-Vis, 2489, Water Corporation, USA). Lycopene was detected at 473 nm and β-carotene at 450 nm. For the photo array (PAD, 2998, Waters Corporation, USA) analysis detection was from 200 to 600 nm. Chromatographic separations were performed on a RP-C30 column (5 μm, 250 × 4.0 mm, YMC Europe, Dinslaken, Germany) connected to a C30 guard column (5 μm, 10 × 4.0 mm, YMC Europe, Dinslaken, Germany) using a flow rate of 0.65 mL/min. The temperature of the column was 25 °C. The mobile phase used in the study 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).

The samples were filtered through a 0.45 mm polyvinylidene fluoride (PVDF) syringe filter ("Millipore", USA) before injection. To quantify lycopene in the

extract samples, a calibration curve was generated using an authentic *all-trans*-lycopene and *all-trans*-β-carotene standard. The levels of *cis*-lycopene isomers are given in *all-trans*-lycopene equivalents. The received data was processed with the Waters Empower software (Milford, USA).

3.4. The extraction of lycopene from tomato by-products using supercritical carbon dioxide extraction and the characterisation of obtained extracts

Plant material preparation

The experiments were performed in the Laboratory of Biochemistry and Technology at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Healthy, ripe, red tomato cv. 'Admiro' F1 were visually selected and harvested. The fruit were washed with tap water, dried and processed. The tomato juice with pulp was separated from seeds and peels. 'Admiro' F1 tomato by-products (peels, vascular tissues, seeds and small amounts of pulp) were freeze-dried using a FD8512S freeze-dryer (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 °C prior to the extraction with SC-CO_2. The typical particle size of the freeze-dried tomato by-product powder obtained in our study was < 0.20 mm. The total lycopene and its *cis*-isomer content in the tomato by-product powder after freeze-drying and in the tomato by-product oleoresin obtained by SC-CO_2 were determined using the HPLC method. The recovery of oleoresin and total lycopene from tomato freeze-dried powder obtained by Soxhlet automated extraction (Behr Labor-Technik, Germany) with the mixture of chloroform and hexane (1:1, v/v) was considered as maximum extraction yield which was later compared with the results obtained using optimized SC-CO_2 extraction. Soxhlet extraction was carried out for 15 hours. The Soxhlet extractor was covered with aluminium foil to prevent oxidation and degradation of valuable carotenoids (especially lycopene). The solvents were removed in a rotary vacuum evaporator (Büchi, Flawil, Switzerland) at 42 °C and the residue was weighed with analytical balances.

Particle size analysis

The particle size distribution of lycopene by-product powder was measured on a particle size analyser (Mastersizer, Hydro 2000S (A), United Kingdom) operating using a laser diffraction method, which is widely accepted as a standard technique. Particle assessment was performed by following the guidance of ISO13320-1 (1999). Water was used as a dispersant for wet analysis, dispersant refractive index was 1.33, and particle refractive index was 1.53.

Supercritical CO_2 extraction

The SC-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 by a surrounding heating jacket. The volume of CO_2 consumed was measured by a ball float rotameter and a digital mass flow meter in standard litres per minute (SL/min) at standard state (P_{CO2}=100 kPa, T_{CO2}=20 °C, ρ_{CO2}=0.0018 g/mL). The process consisted of static (10 min) and dynamic extraction steps. The static extraction time was included in the total extraction time.

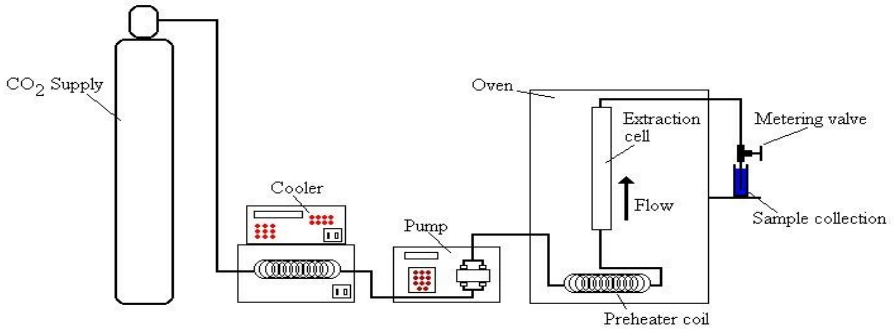


Fig 3.1. The schematic diagram of the SC-CO_2 extraction apparatus

The samples were collected in an amber flask and cooled in an ice bath. The collected extracts were kept at -22 °C before analysis. Lycopene and its *cis*-isomer content in extracts from tomato by-products were determined using HPLC. The amount of extract was determined gravimetrically (±0.01g).

Experiment design

Response surface methodology (RSM) using central composite design (CCD) (Sharif et al., 2014) was employed to determine the effect of temperature (T), extraction time (t) and extraction pressure (P) on the extract (oleoresin) yield and the total yield of *cis*-lycopene isomers in extracts to identify the optimum conditions for total extraction yield and the yield of *cis*-lycopene isomers.

All results were analysed using StatEase Design-Expert 7.0.0 Trial computer software (StatEase Inc., Minneapolis, MN, USA). The design consists of three (T, t and P) (f=3) independent variables with five levels.

The factors and their levels are shown in Table 3.1. The complete design consisted of 20 experimental points (runs 1–20) with 8 factorial points (2^f), 6 axial points (2·f) and 6 centre points (c) (3.1):

$$N = 2^f + 2 \cdot f + c \tag{3.1}$$

where *f* is the number of factors; *c* is the number of centre points.

A full second-order polynomial model of the design is given in Eq. (3.2) and was used to evaluate the yield of total oleoresin and the yield of *cis*-lycopene in oleoresin (response variables, *Y*) as a function of independent variables (*X*), namely,

extraction temperature (°C), extraction pressure (P), extraction time (min), and their interaction (Luengo et al., 2014):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{4} \beta_{ij} X_i X_j \quad (3.2)$$

where Y is the predicted response;

β_0 is a constant (the value of fixed responses at the central point of the experiment);

β_i, β_{ii}, β_{ij} are linear, quadratic and interaction regression coefficients of the model;

X_i and X_j are coded independent variables.

Statistical significance of the model and model variables was determined at the 5% probability level ($p<0.05$). The adequacy of the model was determined by evaluating the lack of fit coefficient and the Fisher test value (F-value) obtained from the analysis of variance. Extractions at every experimental point were performed in triplicate and in random order.

Assessment of extract composition and properties

The main characteristics of SC-CO_2 extract in optimal condition, such as fatty acid composition, the content of tocopherols (the sum of α-, γ-, and δ-tocopherols) and peroxide value of the extract were determined according to the official AOAC methods (AOAC, 1990). Fatty acid composition of the lipid fraction obtained by SC-CO_2 at optimal conditions was analysed by gas chromatography. Fatty acid methyl esters were prepared by using BF_3 catalyst according to the official AOAC method (1995). The amount of extract was determined gravimetrically (±0.01g).

3.5. The stability and isomerisation of lycopene in an oil-based model system during accelerated shelf-life storage

Sample preparation

The experiments were performed in the Laboratory of Biochemistry and Technology at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Fresh tomato (*Lycopersicon esculentum* L.) of the hybrid 'Admiro F_1' (grown in the greenhouses of the Institute of Horticulture) and virgin rapeseed oil (Lithuania) were used for the model system. The tomatoes were red ripe, uniform in size, firm and undamaged.

A model system of lycopene in oil-based food was prepared. Whole washed tomatoes were chopped into pieces and mixed for 3 min into a homogenous mass using an electric blender MMB 2000 UC ("Bosch", Germany). The puree was then mixed by a laboratory stirrer ER-10 ("VEB MLW Prufgerate", Germany) with virgin rapeseed oil using a volumetric ratio of 1:1 (v/v). The temperature of the solution was stable during extraction (20 ± 1°C) (thermostat model 9100, "PolyScience", USA). After 2 hours, the oil phase was separated by a preparative centrifuge ЦРЛ-1 ("Технокom", Russia). The extract was poured into 20 units 2 mL vials, and the extract was divided into five groups. The stability of lycopene-rich oil

extract was investigated during a 200-day storage period. The storage conditions were as follows: 1) refrigerator temperature at 1±1°C in absence of light (FT dark), 2) ambient temperature 20±1°C in natural light (day and night illumination was different) (AT light), 3) ambient temperature 20±1°C in absence of light (AT dark), 4) thermostatically-controlled temperature (37±1°C) in UV irradiation (TT UV), 5) thermostatically-controlled temperature (37±1°C) in absence of light (TT dark). The control sample in our study was lycopene in an oil-based food model system in the zero day (0). The samples were stored in hermetically sealed containers. The control sample and all lycopene oil-based food model samples were prepared for HPLC analysis after storage.

Colour measurements

The colour was measured by a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory Inc., USA). The apparatus (45/0 geometry, illuminant D65, 10 observer) was calibrated with a standard tile (X = 81.3, Y = 86.2 and 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, and the chroma (C) (Equation 3.3), hue angle (h°) (Equation 3.4) and the total colour differences (ΔE) (Equation 3.5) were calculated:

$$C = (a^{*2} + b^{*2})^{1/2} \tag{3.3}$$

$$h^{\circ} = \arctan(\frac{b^*}{a^*}) \tag{3.4}$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{3.5}$$

The L^*, C, a^*, and b^* volumes were measured in NBS units, and the hue angle was measured in degrees from 0 to 360°. An NBS unit is a unit of the USA National Standard Bureau, and it corresponds to one threshold of colour distinction power, i.e. the least distinction in colour that the trained human eye can notice (McGuire, 1992). The colour parameters were processed with the Universal V.4-10 program software. Colour measurements were performed in triplicate.

Storage conditions measurement

The temperature and illumination were recorded using a HOBO Pendant Temperature/Light Data Logger (8K-UA-02-08, "Onset", Finland) at 6-hour intervals and were processed with software HOBOware Pro.

The temperature and the illumination in the storage conditions of the samples during the 200-day test period is shown in Supplement 1.

3.6. The influence of fermentation with lactic acid bacteria on the recovery of carotenoids from tomatoes and tomato by-products

Materials

Tomato var. Cunero and Ronaldo, were obtained from the Lithuanian Institute of Horticulture (Babtai, Lithuania). Raw tomato fruit reference samples were refrigerated for later analysis. After defrosting at 4 °C, tomato samples were homogenized in a blender (Bosch, Stuttgart, Germany) and then analysed. Tomato by-products powder (TP) was obtained from Obipektin AG (Bischofszell, Switzerland) with the certificate of GRAS. The lactic acid bacteria (LAB), *Lactobacillus sakei* KTU05-6 (*L. sakei), Pediococcus acidilactici* KTU05-7 (*P. acidilactici*) and *Pediococcus pentosaceus* KTU05-9 (*P. pentosaceus)* previously isolated from spontaneous Lithuanian rye sourdoughs and selected, were used for the fermentation of tomato and tomato powder (TP) and were obtained from the collection of the Kaunas University of Technology (Kaunas, Lithuania). The LAB were stored at -80 °C and cultured at 30 °C (strain *L. sakei*) , 32 °C (strain *P. acidilactici*), and 35 °C (strain *P. pentosaceus*) for 48 h in MRS broth (CM0359; Oxoid Ltd, Hampshire, UK) used in further experiments.

The LAB strains were propagated in nutrition media (moisture content of 72 %; water activity 0.92): prepared by mixing the extruded rice flour (100 g) (Ustukiu malunas, Pasvalys, Lithuania) and tap water. After the addition of pure LAB cell suspension (5 g, 10.2 $\log_{10}$ colony-forming units (CFU) per g), the mixture was incubated at optimal temperatures for 24 h. For comparison, a control product was prepared using spontaneous fermentation of rice flour without bacterial inoculum at 30 °C for 48 h. Enumeration of LAB was carried out by plating the diluted samples onto De Man-Rogosa-Sharpe (MRS) agar at 30 °C for 48 h. The products obtained after the propagation of individual LAB in rice media were used for fermentation of tomato pulp. The fermented rice product (as medium for LAB grow) was prepared using the individual LAB (20 g) or treated by spontaneous fermentation and were mixed with the homogenized tomato pulp (140 g). The mixture was incubated at appropriate temperatures (30–35 °C) for 48 h. The pH values of the fermented products were measured and recorded by a pH electrode. The total titratable acidity (TTA) was determined according to Sadler and Murphy (2010) and expressed as g per L of citric acid.

Solid state fermentation for TP (sterile, without LAB) was used. Samples were prepared for fermentation by mixing the TP (300 g) with appropriate amounts of water and LAB culture suspension (10 g), containing an average of 10.1 $\log_{10}$ of colony-forming units (CFU) (moisture content of mass 45% w/w, water activity was 0.91). Samples were incubated at 35 °C (*P. pentosaceus*) or 30 °C (*L. sakei*) temperature for 48 hours. Spontaneous fermentation of TP was carried out at 30 °C temperature for 48 hours without LAB starters.

Microbiological analysis

Ten grams of sample were homogenized with 90 mL of saline (0.9%). The suspension was diluted, and 100 mL of each 10^{-4}–10^{-8} solution was grown in MRS

agar. The plates were incubated under anaerobic conditions at 30 °C (for *L. sakei* and spontaneous fermentation), 32 °C (for *P. acidilactici*), and 35 °C (for *P. pentosaceus*) for 72 h. The LAB cell number was calculated and expressed as $\log_{10}$ CFU /g.

Simultaneous determination of L- and D-lactic acid

A rapid and specific Megazyme assay kit for simultaneous determination of L- and D-lactic acid (Megazyme Int., Bray, Ireland) in foods was used as reported by De Lima et al. (2009).

Preparation of ready-to-cook minced meat products

Fresh loin pork was obtained from a local market and used for the preparation of the ready-to-cook minced meat products (RCMP). Meat was minced with a meat grinder (hole diameter of 5.0 mm) (Meissner AG, Biedenkopf-Wallau, Germany), and mixed with the tomato product (fermented with LAB or spontaneously fermented) at selected concentrations (10 and 30 %). Control samples were prepared using minced meat with and without the addition of non-fermented TP. Minced meat samples were covered with plastic film and stored at 4 °C for 24 h until analysis. Non-treated and thermally treated (cooked in water at 100 °C for 10 min) meat samples were subjected to measurements of colour and carotenoids content, and sensory analysis.

Sensory evaluation of ready-to-cook minced meat products enriched with fermented tomato products

Sensory analysis of the ready-to-cook minced meat products (RCMP) was performed according to the ISO 8586-1:1993 (1993) method by fifteen judges using a 10-score hedonic line scale (from 10 (extremely like) to 0 (extremely dislike)). Coded samples were served and water was provided for rinsing between the sensory evaluations of the samples.

3.7. Characterisation of tomato juice and different tomato-based juice blends fortified with isomerised lycopene extract

Preparation of isomerised lycopene extract

Lycopene extract was extracted with supercritical carbon dioxide from dried tomato by-products left after juice preparation. The total lycopene concentration in the extract that was added to tomato juice and juice blends was 11.14 μg/g of extract, of which 65.84% was *cis*-lycopene and 34.16% was all-*trans*-lycopene.

Juice preparation and its enrichment with isomerised lycopene extract

Tomato, apple, carrot, and sweet corn juice was prepared in the Biochemistry and Technology laboratory of Institute of Horticulture (Research Centre for Agriculture and Forestry, Babtai, Lithuania). Prior to processing, tomatoes (“Admiro F1”), apples (“Auksis”), carrots (“Garduolės”) and sweet corn (“Overland”) were thoroughly washed and prepared for juicing. Juice was extracted using Hurom Juicer (Hurom Group Corporation). Then three different tomato-apple juice blends 85:15,

75:25 and 65:35, v/v (TApl15%, TApl25%, TApl35%, respectively), three different tomato-carrot juice blends 60:40, 50:50 and 40:60, v/v (TCar40%, TCar50%, TCar60%, respectively), and three different tomato-sweet corn juice blends 85:15, 75:25 and 65:35, v/v (TCor15%, TCor25%, TCor35%, respectively) were prepared. Before thermal treatment, 5% of isomerised lycopene extract was added to tomato juice and all the juice blends prepared. The control sample was tomato juice with 5% additive of extract with isomerised lycopene. Juices with isomerised lycopene extract were homogenized and pasteurized (10 min at 95±5 °C), then poured to 350 mL glass jars. The pasteurized juices were sealed with metal covers and kept in a dark room at ambient temperatures until analysis (approximately 4 weeks).

Electronic tongue system and sampling procedure

An E-tongue (α-Astree, Alpha MOS Company, France) was employed to classify and characterize the juices. This instrument mainly consists of seven potentiometer chemical sensors (ZZ, BA, BB, CA, GA, HA and JB), a reference electrode of Ag/AgCl, data acquisition system and basic data analysis software. The sensitivity of the seven chemical sensors is different from that of the five tastes (sourness, saltiness, sweetness, bitterness and savouriness). Experiments were carried out with filtrated juice to avoid the influence caused by solid particles. The amount of each juice sample was 80 mL to ensure that the sensors could be fully immersed in the liquid. The measurement time was set to 120 s for each sample, which was long enough for the sensors to reach stable signal values, and the sensors were rinsed for 10 s using deionized water to minimize and correct the drift of sensors. Measurements were carried out at 20±3°C.

The concentration of ascorbic acid

The concentration of ascorbic acid (vitamin C) was determined by the titrimetric method using a 2,6-dichlorophenolindophenol sodium salt solution (AOAC, 1990); chloroform was used for intensely coloured extracts.

The total soluble solids

The total soluble solids (TSS) were determined using a digital refractometer (ATAGO PR-32, Atago Co., Ltd., Tokyo, Japan).

The titratable acidity

The titratable acidity (TA) was determined by titration with 0.1 N NaOH to a pH 8.2 end point and expressed in percent of citric acid equivalents.

Sensory assessment

Regular consumers (32 people) of fruit and/or vegetable juices participated in consumer evaluation session. All consumers were between 20 and 65 years of age. They used a hedonic scale to evaluate the samples (5 – the highest acceptability, 1 – the lowest acceptability). In addition, the panellists were asked to indicate the defect, which they had observed in the sample, if a score of 3 points or lower was given.

3.8. Measurement of anti-inflammatory and antiproliferative properties of extract with isomerised lycopene

The analysis of anti-inflammatory and antiproliferative properties of lycopene isomers extract was carried out at the Institute of Neuroscience of Medical Academy, Lithuanian University of Health Science.

Preparation of lycopene isomers extract

Lycopene crystals (10 mg) were dissolved in 20 mL of tetrahydrofuran and thermostated for 12 h in 37 °C ± 1°. Followed by the solvent evaporation in a rotary evaporator and the removal of solvent residue under the gentle nitrogen-flow, the lycopene content and isomerisation ratio was measured with HPLC. The samples were stored at -80 °C until further use or analysis. The lycopene isomer mixture (*trans/cis* 4:6) was evaluated.

Cell culture

Murine macrophage cells of J774 and rat glioblastoma C6 cells (Lines Service GmbH (Vokietija) were used for the experiments. Murine macrophage cells of J774 were maintained in DMEM + 10% fetal calf serum + penicillin (100 U/mL) / streptomycin (100 μg/mL) medium at 37 ºC in a humidified atmosphere containing 5% of CO_2. Glioblastoma C6 cells were maintained in DMEM + 10% fetal calf serum + penicillin (100 U/mL) / streptomycin (100μg/mL) and glutamine (2 mM) medium at 37 ºC in a humidified atmosphere containing 5% of CO_2. Every 3–4 days, the cells were subcultured.

Measurement of hydrogen peroxide production in the macrophage culture

In the incubation model, a murine macrophage J774 cell suspension (3 x 10^5 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 (0.2–1.2 μM) were added to the cell culture and incubated in a thermostat (at 37 ºC in a humidified atmosphere containing 5% of CO_2). 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 ºC. Hydrogen peroxide production in the macrophage culture was measured fluorimetrically using an Ascent Fluoroscan plate reader (Thermo Fisher Scientific, Waltham, MA) at the excitation of 544 nm and an emission of 590 nm. The production of hydrogen peroxide was obtained by stimulating NADPH oxidase with PMA (10 μM) in a medium containing 1 μM of Amplex Red and 10 U/mL of horseradish peroxidase. Horseradish peroxidase uses Amplex red as an electron donor for the reduction of hydrogen peroxide to water; the reaction product resorufin is a colourful and fluorescent component. The fluorescence signal was evaluated according to the calibration curve of hydrogen peroxide (H_2O_2, nmol). For the evaluation of direct measurement of lycopene extract activity several concentrations of 0.2 and 1.2 and 7 μM lycopene extract were selected.

A similar measurement procedure was performed for the direct evaluation of lycopene activity. The macrophage culture in measurement wells was directly

affected by different concentrations of lycopene extract (0.2, 1.2, and 7 μM) and the production of hydrogen peroxide was measured fluorimetrically in a period of 15 min.

Measurement of intracellular reactive oxygen species in a glioblastoma culture

Reactive oxygen species in a rat glioblastoma C3 cell culture were measured fluorimetrically (excitation of 485 nm and an emission of 530 nm) using DCHF-DA dye. DCHF-DA easily diffuses in to the cells and is deacetylated by cell esterases in to DCHF. Reactive oxygen species e.g. cell-originated hydrogen peroxide, oxidize DCFH into a fluorescent compound DCF. The intensity of fluorescence is proportional to the originated amount of H_2O_2 in cells. The cell suspension (3×10^4 cells/200μL) added to 96-well plates (each with 0.2 mL of medium) and loaded with 10 μmol/L of DCHF-DA for 30 min at 37ºC in a dark place is treated with different concentrations of lycopene extract (0.05, 0.1, 0.6, 1.2, 3 and 6 μM) (dissolved in DMSO) for 0.5–3 h. Then the lycopene extract is carefully washed out with PBS buffer and the generation of ROS is measured using a fluorimeter. The data was compared with the control cell group (untreated with lycopene extract). The used DMSO concentrations had no effect on the production of ROS.

Evaluation of cell viability

Cell viability was measured using an MTT assay. MTT, a yellow tetrazole, depending on the redox status of the living cell is reduced and turns into a colourful (purple) compound formazan, therefore the colour change of MTT in an indicator of enzymatic oxidation reduction reactions determined by the activities of various dehydrogenases. The amount of originated formazan is directly proportional to the number of viable cells. The activity of dehydrogenases (and cell viability) was evaluated using an MTT test. The glioblastoma cell culture was treated with different concentrations of lycopene extract (0.025, 0.05, 0.1, 0.2, 0.6, 1.2, and 3 μM) for 24 h. After the period, the medium was removed; cells were loaded with 0.5 mg/mL solution of MTT and incubated for 1 h. After the incubation, the light absorption was measured at 570 nm wavelength. The reduction of control cells (untreated with lycopene extract) was equated to 100%.

Statistical analysis

All experiments were carried out and each collected sample was analysed in triplicate. The mean values and standard deviations of the experimental data were calculated using MS Excel 2010 software and SPSS 20 Software (SPSS Inc., Chicago, USA). The mean values were further compared using Tukey's test, and the differences were considered to be statistically significant when $p \leq 0.05$. Principal component analysis (PCA) was used to classify juice samples according to their similarity in taste.

RSM using CCD was applied to determine the optimal extraction conditions by SC-CO_2 for maximizing the yield of total oleoresin and the amount of *cis*-lycopene in the extracts. Analysis of variance (one-way ANOVA) was used to test the differences among group means, followed by the Duncans' *post hoc* test.

Statistical significance of the model and model variables was determined at the 5% probability level ($p<0.05$). The adequacy of the model was determined by evaluating the "lack of fit" coefficient and the Fisher test value (F-value) obtained from the analysis of variance. Extractions at every experimental point were performed in duplicate and in random order.

4. RESULTS AND DISCUSSION

4.1. A qualitative and quantitative analysis of lycopene isomers and β-carotene using high-performance liquid chromatography

Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds from complicated matrices. The most popular and reliable system for the separation of carotenoids is high performance liquid chromatography (HPLC) (Mercadante, 2007). The objective of this study is to develop a HPLC–DAD-sensitive method for qualitative and quantitative determination of lycopene, lycopene isomers, and β-carotene in different systems from fresh tomato and tomato-based food products to model food systems and fermented products.

Experiments during the development stage showed that better chromatographic separation was achieved by using the C30 (30 bonded silica-based reversed-phase column) stationary phase and this system is suitable for the separation of all *trans*-lycopene from any *cis*-lycopene (Motilva et al., 2014; Amorim-Carrilho et al., 2014). The C30 column provided sufficient resolution of lycopene and resolved structurally similar lycopene isomers. Different lycopene isomers were eluted as single chromatographic peaks. The problem was the structural identification of lycopene and its isomers.

According to the literature, a highly effective structural assignment is coupling of LC to MS. However, lycopene and its *cis*-isomers are non-polar carotenoids and cannot be ionised by standard electrospray ionisation. The main alternative is atmospheric pressure chemical ionisation (APCI). However, the APCI technique is only suitable for initial identification of different carotenoids but is not suitable for the identification of different carotenoid isomers. According to the literature, APCI only differentiates between β-carotene and lycopene (Heymann et. al., 2013). The UV-Vis spectrum of carotenoids and lycopene is characteristic, which can serve as a basis for identification and quantification (Rodriguez-Amaya, 2001; Shi and Maguer, 2000). The HPLC with photodiode array detector that provides the visible absorption spectra of the separated carotenoids on-line could be an alternative for identifying the carotenoid isomers (Rodriguez-Amaya, 2010).

In this study, a broad variety of analytical methods and chromatographic parameters was employed for the detection of β-carotene, lycopene and its *cis*-isomers, and sufficient separation of the relevant isomers was achieved in raw tomato materials and their products. Selectivity of the *cis*-lycopene isomers was suitable as the mean of a measure of how well an analyte can be determined in a complex mixture without any interferences, in order to separate lycopene from β-carotene. Moreover, the method has selectivity for the lycopene *cis*- and *trans*-isomers.

Figure 4.1 shows the chromatogram obtained by HPLC-DAD at 473 nm. The absolute configuration of different carotenoids and their isomers was verified by various approaches. A comparison for the analysis of the commercially available standards retention times led to the identification of *all-trans*-β-carotene (peak No.

1) and *all-trans*-lycopene (peak No. 6). The lycopene isomers were assigned based on their spectral characteristics by HPLC-DAD analysis.

According to literature, the Q ratio was used to characterize the *cis*-lycopene isomer peaks (Table 4.1). In comparison to the literature data (Schierle et al., 1997; Heymann et al., 2013), a more central *cis*-bond leads to a higher *cis*-peak to give a higher Q ratio. Apart from the peak corresponding to *all-trans*-lycopene, other five peaks (peak No. 2–5 and 7) were identified (*15-cis*-lycopene (peak No. 2), *13-cis*-lycopene (peak No.3), *9-cis*-lycopene (peak No. 4), *7-cis*-lycopene (peak No. 5), *5-cis*-lycopene (peak No.7)). The experiment gave the highest Q-ratio for *13-cis*-lycopene (0.53), followed by *15-cis* (0.31), *9-cis* (0.14), *7-cis* (0.13) and *5-cis* (0.68). The other peaks in the chromatogram (from 20 to 50 min) could not be clearly attributed due to the lack of authentic standards, but compared with previous literature and UV/Vis data for our study, these signals were suggested to be di- or poly-*cis*-isomers (Lee and Chen, 2001).

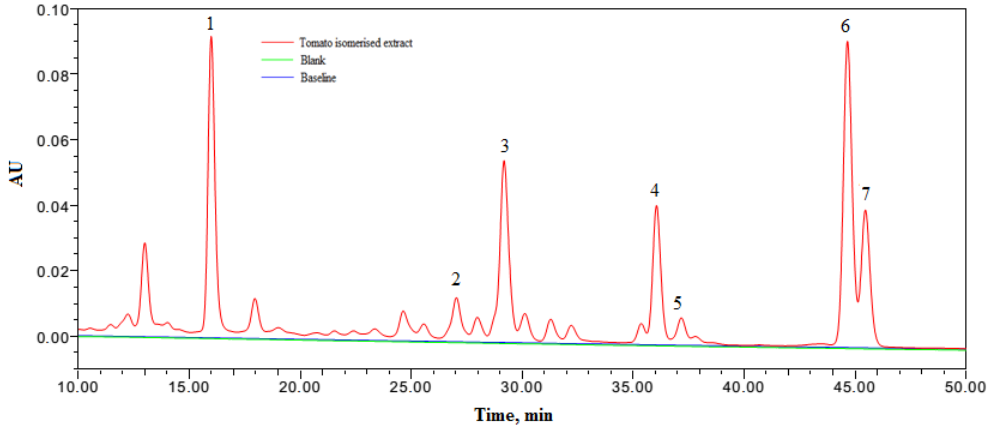


Fig.4. 1. HPLC with UV-Vis detection of 473 nm showing the separation of different lycopene isomers and *β*-carotene of the tomato isomerised extract and the basic line. Numbers refer to the identified compounds: 1 – *trans*-β-carotene; 2 – *15-cis*-lycopene; 3 – *13-cis*-lycopene; 4 – *9-cis*-lycopene; 5 – 7-*cis*-lycopene; 6 – *trans*-lycopene; 7 – *5-cis*-lycopene

In summary, the presented identification of β-carotene, lycopene and lycopene isomers based on the combination of authentic standards and UV-Vis data fully complies with the literature and can be used for the following qualitative and quantitative analyses of lycopene, lycopene isomers, and β-carotene in different food systems and extracts.

Table 4.1. Photodiode array data from HPLC peaks of a tomato extract

HPLC peak[a]	Lycopene (LYC) isomer	Photo array detector data of HPLC peaks				
		Relative absorption (λ) of *cis*-peak[c] (nm)	Absorption maxima[b] (nm)			
1	*trans*-β-CAR	–	–	274.9	<u>451.6</u>	477.1
2	*15-cis*-LYC	**0.31** (0.38[1]; 0.57[2])	359.6	440.7	<u>462.5</u>	489.3
3	*13-cis*-LYC	**0.53** (0.55[1]; 0.48[2])	359.6	440.7	<u>462.5</u>	495.4
4	*9-cis*-LYC	**0.14** (0.12[1]; 0.26[2])	360.6	440.7	<u>466.2</u>	497.8
5	*7-cis*-LYC	**0.13** (0.12[1]; 0.19[2])	360.6	440.7	<u>462.5</u>	497.8
6	*trans*-LYC	**0.067** (0.06[1]; 0.06[2])	360.6	445.5	<u>472.3</u>	502.7
7	*5-cis*-LYC	**0.068** (0.06[1]; 0.11[2])	360.6	443.3	<u>472.3</u>	502.5

[a]Peak numbers shown in the chromatogram (Fig. 4.1).
[b]Main absorption maxima are underlined.
[c]Relative absorption (λ) of *cis*-peak (bolded) means the absorption at the subsidiary peak (at approx. 360 nm) divided by the absorption at λ_{max}. Comparatively high relative absorptions, as recorded for peaks, indicate lycopene *cis*-isomers with a more-central *cis*-double-bond (e.g. *13-cis*-lycopene). In contrast, low relative absorptions are characteristic of more-terminal *cis*-double-bonds.

A single-laboratory validation study was conducted for a reversed phase high performance liquid chromatography (RP-HPLC) method for the determination of total lycopene and all-*trans*-lycopene in tomato fruits, tomato-based food products and oil-based food systems. The method is suitable for determining all-*trans*-lycopene and total lycopene. The term total lycopene comprises all-*trans*-lycopene and the major *cis*-isomers, *5-cis*-lycopene, 7-*cis*-lycopene, *9-cis*-lycopene, *13-cis*-lycopene and *15-cis*-lycopene.

The validation was carried out to single-laboratory validation with an analytical method based on the ICH recommendation and literature (Muller et al., 2008; Dias, Camões, Oliveira, 2008; Silva et al., 2011) under the best conditions by assessing reference 1–7 peak areas in the chromatogram (Figure 4.1). Several standard assay validation parameters were chosen: specificity (the selectivity of the method), precision, the limit of detection (LoD), the limit of quantitation (LoQ) and linearity.

The selectivity of the method for main peak identification and purity was evaluated based on the comparison of the retention times and UV-Vis spectra of the analytes with the standard compounds and isomerised lycopene standard to *cis*-lycopene isomers. The identified compounds (*all-trans*-lycopene, *all-trans*-β-carotene, and *cis*-lycopene isomers) in extracts were confirmed by adding the

standard compound in the same extract and monitoring the change in the peak shape and UV-Vis spectral characteristic.

The limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value (Table 4.2). The ratio of the peak height to baseline noise (signal-to-noise ratio) used for the estimation of the quantitation limit was 10:1. The determined quantitations limit was 0.92 μg/100mL and 0.81 μg/100mL for all-*trans*-lycopene and β-carotene, respectively. The linearity of the HPLC method was determined to compare the dependence of peak plot area change on the analyte concentration. The estimated regression coefficients (R^2) of calibration equations were greater than 0.99 which proves the linearity of the quantitative determination method (Table 4.2). The calculated detection limit values of the analytes of interest ranged from 0.22 μg/mL to 0.41 μg/mL, the quantitative limit ranged from 0.65 μg/mL to 0.83 μg/mL (Table 4.2).

Table 4.2. Characteristics of the quantitative evaluation of analytes

Compound	LoD[a] (μg/mL)	LoQ[b] (μg/mL)	Confirmed linearity range (μg/mL)	Calibration equation	R^2 [c]
All-*trans*-lycopene	0.23	0.71	0.625–100	$y=6.53\times10^4x+2.57\times10^3$	0.999
All-*trans*-β-carotene	0.41	0.83	0.625–100	$y=5.46\times10^4x+2.67\times10^3$	0.998
15-cis-lycopene	0.22	0.65	0.625–100	$y=6.53\times10^4x+2.57\times10^3$	0.999
13-cis-lycopene	0.31	0.70	0.625–100	$y=6.53\times10^4x+2.57\times10^3$	0.999
9-cis-lycopene	0.25	0.67	0.625–100	$y=6.53\times10^4x+2.57\times10^3$	0.999
7-cis-lycopene	0.27	0.71	0.625–100	$y=6.53\times10^4x+2.57\times10^3$	0.999
5-cis-lycopene	0.28	0.68	0.625–100	$y=6.53\times10^4x+2.57\times10^3$	0.999

[a] Limit of detection, [b] Limit of Quantitation, [c] Regression coefficient

The precision of the HPLC method was assessed on the basis of two parameters: repeatability and intermediate precision. The repeatability of the results was evaluated on the basis of the results of consecutive analyses performed within the same day (6 consecutive analyses of a mixture of standards at 3 different concentrations performed within the same day). The percent relative standard deviation (%RSD) of the repeatability of the method determined according to the peak area was 1.20% and 1.47% (*all-trans*-lycopene and all-*trans*-β-carotene), respectively. The intermediate precision of the results was assessed based on the results of analyses performed within 3 different days (6 consecutive analyses of a mixture of standards at the mean concentration performed within the same day; 18 analyses in total), and its %RSD for the peak area ranged from1.95 to 2.41 %. The

extraction after the extraction of the freeze-dried tomato extract was performed. A set of 6 tetrahydrofuran extracts was produced from the same fresh tomato sample and was analysed by applying the developed HPLC method. The estimated %RSD for the peak area did not exceed 3.54%. The obtained results describing the precision of developed HPLC method are summarized in Table 4.3.

Table 4.3. Precision of the HPLC method for the quantitative evaluation of main carotenoids

Compound	Relative deviation (%) for the peak area		
	Intraday[a]	Interday[b]	Extraction after extraction
All-*trans*-lycopene	1.20	1.95	2.91
All-*trans*-β-carotene	1.47	2.36	3.54
15-cis-lycopene	1.12	2.14	2.80
13-cis- lycopene	1.05	2.32	2.78
9-cis- lycopene	1.18	2.41	2.83
7-cis- lycopene	1.15	2.38	2.85
5-cis-lycopene	1.23	2.18	2.76

[a]Repeatability.
[b]Intermediate precision.

The HPLC method was validated with respect to precision, linearity and selectivity and confirms the value of the methodology to analyse the suitability of lycopene, lycopene isomers and β-carotene. This procedure is applicable for further research.

4.2. A study of supercritical carbon dioxide extraction and optimisation of extraction parameters of non-polar fractions from tomato by-products

The majority of scientists agree that the SC-CO_2 extraction has established the environmental soundness of the technology and complimentary safe solvent for extracting food, cosmetic and pharmaceutical industry grade and bioactive components from agricultural products. Some research studies demonstrated the successful extraction of lycopene from tomato by SC-CO_2 extraction (Kassama et al., 2008; Zuknik, Norulaini and Omar, 2012). Although all the studies focused only on the effect of various SC extraction parameters, such as temperature, pressure, CO_2 flow rate, extraction time on extraction yield and total lycopene yield (Kassama et al., 2008; Perretti et al., 2013; Silva et al., 2014; Yener et al., 2015; Azabou et al., 2016). Nevertheless, most of them deal with the extraction of total lycopene content, not distinguishing the different composition of lycopene isomers. However, different parameters of SC extraction can have individual or combined effects not only on the extraction rate and lycopene yield, but are also important factors on the bioactivity and composition of the obtained extracts. As mentioned in the literature review, the studies attribute higher bioavailability to the *cis*-lycopene isomers. A comparison of the reported results involving SC-CO_2 extraction of lycopene from tomato is rather complicated due to the differences in the applied extraction conditions and tomato-derived preparations as the sample source. In addition, the concentrations of the

obtained extraction yield and lycopene have been expressed in different ways, e. g. based on a dry or fresh weight of sample.

One of the objectives of this study is to optimize the parameters of supercritical carbon dioxide (SC-CO_2) extraction and their interaction for the isolation of non-polar tomato by-product fraction (oleoresin) and *cis*-lycopene isomers by RSM using CCD. Oleoresin in this study is defined as the non-polar fraction including carotenoids from tomato by-products after SC-CO_2 extraction. The lycopene extraction procedure was optimized with the aim of obtaining an extract with the maximum concentration of *cis*-isomers with the possibility of having an isomeric composition valued for its biological functions, as mentioned in literature.

Particle size analysis of tomato by-products

Raw material pretreatment is important in order to significantly increase the yield of extract and biologically active components (Baysal et al., 2000, Ciurlia, Bleve, Rescio, 2009, Vasapollo et al., 2004). For example, drying and grinding processes influence the physical properties and quality of raw material. As mentioned in the literature review, particle size significantly influences the yield of extract. In this study, particle size was determined using the wet analysis method. Distilled water was chosen as a dispersant because particles are not soluble in water, while wetting the particles by water is rather efficient. The parameters of the particle size are given in Table 4.4 and the particle distribution is shown in Figure 4.2. Tomato by-product powder exhibited a monomodal repartition; centred on 90–160 μm (Figure 4.2).

Table 4.4. Particle size parameters of tomato by-products sample[a]

Sample	d (0.1)	d (0.5)	d (0.9)	D [4,3]	D [3,2]
Tomato by-product powder particle <200 μm	40.5±1.66	315.5±8.27	849.1±23.34	388.0±8.90	94.7±3.63

[a] The values are expressed in terms of percent (%) as mean ± standard deviation of six determinations. D [4, 3] – volume weighted mean, D [3, 2] – surface weighted mean, d(0.1) – 10% of the particles are smaller than this diameter, d(0.5) – half of the particles are smaller or larger than this diameter, d(0.9) – 90% of the particles are smaller than this diameter.

Kaur et al. (2008) evidenced that the increase in particle size from 0.05 to 0.43 mm did not significantly vary the yield of lycopene. Sabio et al. (2003) verified that the extraction of lycopene from tomato wastes decreased at very small particle sizes (0.08 mm). Strati and Oreopoulou (2014) suggested, through the optimization of extraction parameters with response surface methodology, that the optimized particle size for recovery of carotenoids from tomato waste was 0.56 mm. Therefore, previously reported values are rather variable and difficult to compare.

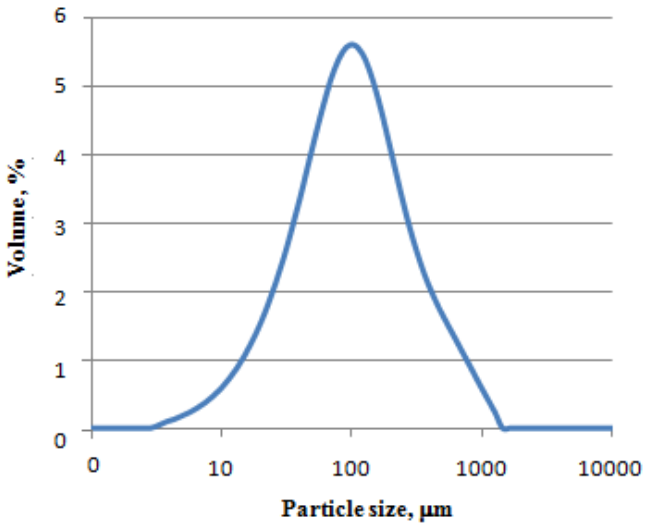


Fig.4.2. Particle size distribution of freeze-dried tomato by-product powder

Taking into account the measured characteristic of the particle size obtained in our study (< 0.20 mm) as well as literature data (0.05–0.56 mm) which suggested that the tomato by-products powder was suitable for SC extraction optimization because numerous experimental results have indicated that smaller particles sizes (0.05–0.56 mm) are ideal for extraction processes (Machmudah et al., 2012, Nobre et al., 2009).

Optimisation of supercritical carbon dioxide extraction parameters

The optimization of extraction parameters for extracting tomato by-products would provide valuable data for process design and industrial scale-up applications. The use of concentrated carotenoid extracts from red-ripe tomato by-products in traditional foods can improve the functional properties of the product while increasing the efficiency of industrial red-ripe tomato processing. It is very important to determine the optimal conditions (temperature, pressure, extraction time) for the extraction of non-polar fraction from tomato by-products using SC-CO_2 and to test the optimised extraction parameters for the determination of total extraction yield and concentration of *cis*-isomers in the isolated fractions.

The levels of variables used in this experiment were determined based on preliminary experiments and literature data. As reported in previous studies, the change of pressure in the range of 20 to 25 MPa have shown insignificant difference in the concentration of lycopene in the extract from tomato by-products (Baysal et al., 2000). The authors of the study found that the highest yields of lycopene were extracted at 40 MPa and suggested that lycopene recoveries could be improved by extractions up to 40 MPa. Similarly, Baysal et al. (2000) and Egydio et al. (2010) studies showed that the highest lycopene yield was obtained at 35 MPa pressure. Shi et al. (2009) also found that the highest pressure used in their study, 35 MPa, led to the highest yield of lycopene at 75 °C temperature. In the SC-CO_2 extraction of

lycopene, the results of numerous investigations indicate the extraction temperature (40–100 °C) to be the most important parameter in the extraction of lycopene from various fruit matrices (Reverchon, De Marco, 2006). With reference to Baysal's et al. (2000) results, the extraction of lycopene by SC-CO_2 indicated that the most suitable extraction time was 120 min, instead of 60 or 180 min. A 60 min extraction may not suffice for the maximum amount of lycopene to be dissolved in the solvent.

The preliminary evaluation of SC-CO_2 extraction kinetics in our study was performed at 37.5 MPa and 55 MPa of pressure and at temperatures of 40, 60, and 80 °C. It was obvious that the majority of the extract was isolated during the first 120 min of dynamic extraction time (Fig. 4.3). The extraction curves reached a plateau at a lower pressure (37.5 MPa) after 160 min. However, at 55 MPa, the extract yield continuously increased up to 180–240 min of processing time. Therefore, the 120–240 min range of dynamic extraction time was selected for optimising the experiment.

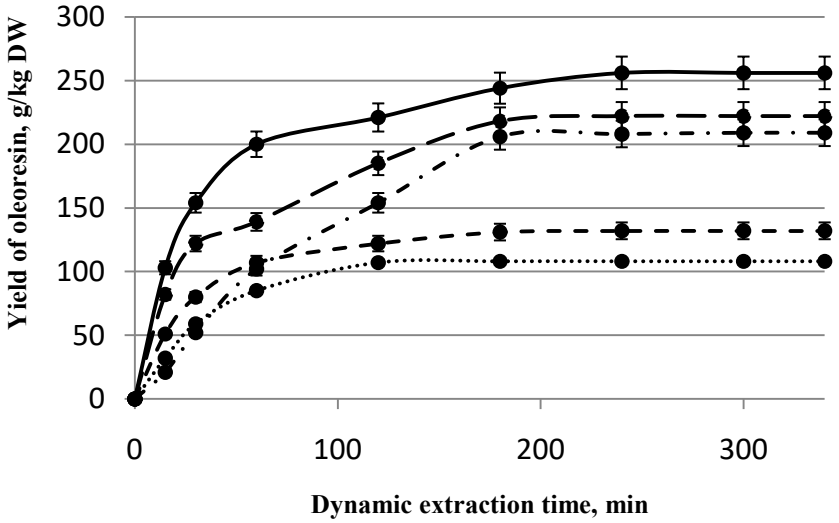


Fig. 4.3. The influence of extraction time on the yield of oleoresin from tomato by-product obtained under different extraction conditions: 80 °C and 55 MPa; 60 °C and 55 MPa; 40 °C and 55 MPa; 80 °C and 37.5 MPa; 40 °C and 37.5 MPa

RSM using CCD was applied to determine the optimal extraction conditions by SC-CO_2 for maximizing the total yield of oleoresin and *cis*-lycopene in the extracts. The RSM has been demonstrated to be an effective and powerful statistical method for studying the relationships between measured responses and independent factors. This method could reduce the number of experimental trials and investigate the critical processes with the correlations between factors that can be used for process optimizations. The experimental design provides the possibility to evaluate interactions between factors, in this case, pressure, temperature and time (static and

dynamic), while limiting the number of experiments. The matrix for the CCD optimization experiment is summarized in Table 4.5.

In this study, RSM using CCD was used to optimize pressure (X_2), temperature (X_1) and extraction time (X_3) on the yield of non-polar fraction and *cis*-lycopene isomers in the obtained extract. The experimentally obtained extract yield for the total oleoresin from tomato by-products and *cis*-lycopene isomers in oleoresin is shown in Table 4.5 A.

Response surface plots showing the effect of extraction temperature, pressure, and extraction time on non-polar tomato by-product fraction (oleoresin) yield (g/kg DW) are presented in Figure 4.4 (a–c). The graphs were obtained by keeping one of the variables constant (coded as zero level), while varying the other two variables and predicting the response. The interaction between different parameters and their importance on the extract yield is shown in 3-D graphs (Fig.4.4).

The 3-D graphs (Fig. 4.4) clearly indicate how different parameter values influence the recovery of obtained non-polar extracts in the non-polar fraction. The yield of total oleoresin from tomato by-products (by Soxhlet extraction) was 322±5 g/kg (DW); this result is comparable with the results of Machmudah, Winardi, Sasaki, Goto, Kusumoto, and Hayakawa (2012) (340 g/kg).

The suggested optimal conditions of the model for obtaining the highest yield of oleoresin (251.2 g/kg DW) are: the temperature of 73.9 °C, the pressure of 53.7 MPa and the time of 155 min. Under these conditions, 78% of oleoresin could be extracted, whereas 22% of the oleoresin remains both in the pipeline and the solid matrix.

Table 4.5. Factors selected as independent variables for the optimization of SC-CO_2

Symbols	Independent variables	Coded levels				
		-1.682	**-1**	**0**	**1**	**1.682**
X_1	Extraction temperature (°C)	26.36	40	60	80	93.64
X_2	Extraction pressure (MPa)	8.07	20	37.5	55	66.93
X_3	Extraction duration (min)	79.09	120	180	240	280.91

The major fatty acids (FA) in tomato by-product extract isolated by SC-CO_2 were polyunsaturated linoleic acid (54±2 %), followed by monounsaturated oleic (21±1%) acid, whereas the polyunsaturated α-linolenic acid constituted only 3±0.2%. The content of saturated fatty acids constituted of palmitic 11±0.6% and stearic acid 5±0.3%. The other FAs were found in remarkably lower amounts, 0.08–0.26 % (elaidic, margaric, gadoleic, myristic, behenic, γ-linolenic, lignoceric, palmitoleic and arachidic). According to the literature, lipids altered the antioxidant effectiveness and stability of lycopene due to lipid peroxidation, therefore the separation of tomato lipids and carotenoids is reasonable. Previous literature shows that tomato seed oil has high thermal stability with an excellent physicochemical profile after heating (de Castillo, Gomez-Prieto, Herraiz, Santa-Maria, 2003). Antioxidants, such as tocopherol, are likely the most significant contributors to the high thermal stability (Shao, Liu, Fang, Sun, 2015). The composition of

antioxidatively active tocopherols (the total concentration of isomers) was examined in this study and the concentration of the tocopherols was 3.01±0.04 g/kg. The peroxide value of freshly extracted oil was 7.5 ± 0.4 meq/kg.

Table 4.5 A. Fully coded CCD and the results obtained for total oleoresin (g/kg DW) and the yield of *cis*-lycopene isomers in oleoresin (g/kg)

Experiment No.	X_1	X_2	X_3	Yield	
				Total oleoresin* (g/kg DW)	*Cis*-lycopene isomer yield in oleoresin* (g/kg)
1	40	20	120	112.2±4.6	79.2±1.9
2	80	20	120	167.1±3.4	43.4±1.0
3	40	55	120	122.3±2.1	79.9±1.2
4	80	55	120	248.1±4.7	54.8±0.5
5	40	20	240	119.2±2.8	80.2±0.4
6	80	20	240	192.1±2.5	44.1±0.2
7	40	55	240	132.1±4.5	80.5±0.9
8	80	55	240	256.1±4.2	56.4±0.3
9	26.36	37.5	180	108.0±3.1	48.3±0.5
10	93.64	37.5	180	252.1±3.9	24.3±0.5
11	60	8.07	180	131.9±2.0	61.0±1.4
12	60	66.93	180	213.5±3.5	85.9±1.4
13	60	37.5	79.09	163.2±2.4	60.4±1.1
14	60	37.5	280.91	214.3±4.5	75.5±1.0
15	60	37.5	180	204.2±2.8	71.4±0.9
16	60	37.5	180	197.2±2.7	72.3±0.7
17	60	37.5	180	199.5±3.3	71.9±0.6
18	60	37.5	180	196.4±2.0	72.1±1.0
19	60	37.5	180	208.1±2.5	71.5±0.5
20	60	37.5	180	202.1±3.1	71.0±0.6
S_p**				3.0	1.8

*Values are represented as a mean ± standard deviation (n = 3)
** Pooled standard deviation

According to Machmudah et al. (2012), the fatty acids are extracted with the pigments in the sample and co-extraction may occur. The fatty acids from tomato seeds in the sample may increase the solubility of the pigments extracted from the sample, including lycopene. According to this hypothesis, all cases in which lycopene is extracted from tomato by-products containing tomato seeds also contain co-extracted fatty acids, thus contributing to the solubility of lycopene in the SC-CO_2.

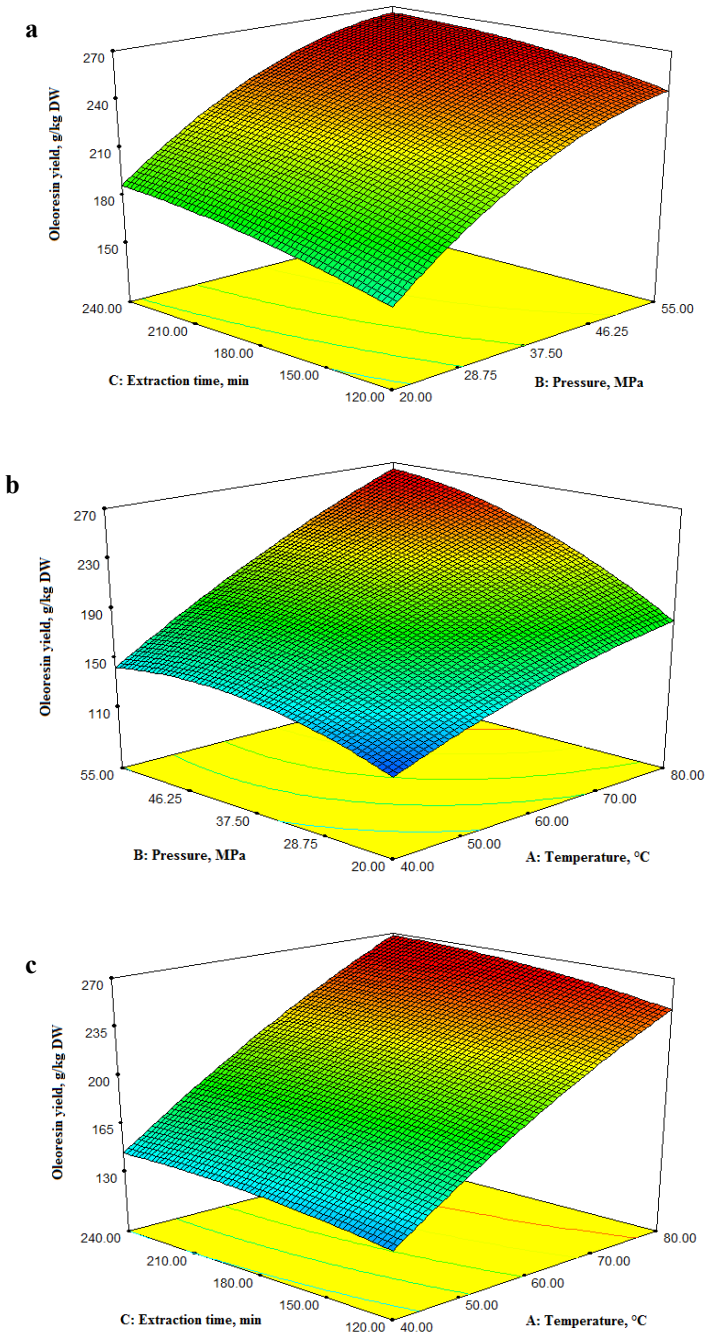


Fig. 4.4. 3D response surface plots of SC-CO_2 showing the effects of independent variables on the extraction yield of tomato by-product oleoresin: (a) the effect of extraction time and pressure; (b) the effect of extraction time and temperature; (c) the effect of extraction pressure and temperature

As mentioned before, some studies concerning the supercritical CO_2 extraction of lycopene from tomato by-products have already been published, such as those of Machmudah et al. (2012), Perretti et al. (2013), Rawson et al. (2012) and Shi et al. (2009). However, there was a large variation in their results. Therefore, in most of these studies, the effect of the content of lycopene in the pre-treatment of the samples is not considered. To our knowledge, all studies dealing with supercritical fluid extraction of lycopene from tomato by-products recovery only used traditional drying methods (infrared radiation drying, convection drying, etc.). It has been shown that the supercritical extraction of compounds from dried tomato by-products results in a low extraction recovery of lycopene. It is possible that lycopene is enclosed into the cell matrix during dehydration process; in agreement to this, the solvent penetration and lycopene dissolution could be complicated. The recovery of lycopene from tomato by-products varies from 33 to 53 % (Kassama et al., 2008; Machmudah et al., 2012; Shi et al., 2009) and all these studies used organic co-solvents.

The carotenoid content of tomato by-products collected from a commercial tomato processing plant may vary, depending mostly on the tomato variety, on the industrial processing methods and technological parameters. In a recent study (Kalogeropoulos et al., 2012), the major carotenoid found in tomato processing waste was lycopene. In our study, the total amount of lycopene in freeze-dried tomato by-product powder from Soxhlet extraction was 158.3±0.12 g/kg (DW).

Only the effect of some SC extraction parameters on the total yield of lycopene and carotenoids from tomatoes and tomato by-products has been studied (Lenucci, et al. (2010), Perretti et al. (2013), Azabou et al. (2016)). Additionally, most studies focused on the amount of total lycopene extracted, and these studies have not characterized the lycopene isomer composition, their extract yield or the extraction efficiency.

The lycopene extraction procedure was optimized with the aim of obtaining an extract with the maximum concentration of *cis*-isomers with the possibility of having an isomeric composition valued for its biological functions. In our study, the extraction temperature has to be fixed between 35 and 80 °C. Obviously, the extract yield increased with temperature, but the total *cis*-lycopene isomers in the extract followed the opposite trend. When the extraction temperature changed from 40 to 60 °C and the pressure changed from 20 MPa to 55 MPa, the extract composition differed ($p < 0.05$). However, when the temperature further increased from 60 to 80 °C, the content of *cis*-lycopene in the obtained extract decreased (Fig. 4.5.). At the temperature of 80 °C, *cis*-lycopene (as well as total lycopene) in the oleoresin may be degraded through oxidation, whereas isomerisation increased at 40 and 60 °C. The results of this study agree with previous reports (Lambelet, Richelle, Bortlik, Franceschi, Giori, 2009), which investigated the thermal stability and isomerisation of lycopene at 20, 25, 37, 50, 75 and 100 °C.

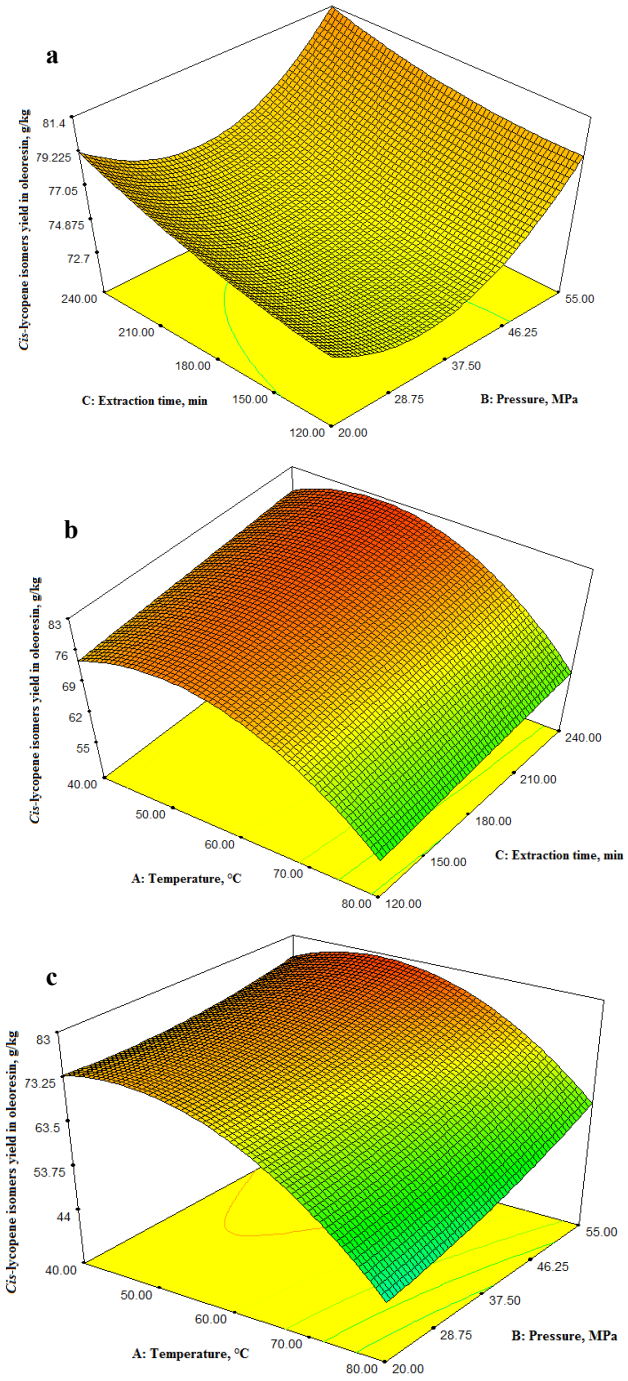


Fig. 4.5. 3D response surface plots of SC-CO_2 showing the effects of independent variables on the total yield of *cis*-lycopene isomers in the obtained oleoresin: (a) the effect of extraction time and pressure; (b) the effect of extraction time and temperature; (c) the effect of extraction pressure and temperature

The change in the amount of lycopene in the extracts was mainly influenced by the lycopene content and proportions of the lycopene isomers. If lycopene is isomerised, the proportion of lycopene isomers changes and then further alters the extract composition. The main geometrical lycopene isomers were *9-cis* and *5-cis*. The optimal extraction process parameters of *cis*-lycopene isomers are a 52 °C temperature, a 55 MPa pressure and a 180 min extraction time (Table 4.5 A). The total content of *cis*-lycopene isomer in the SC-CO_2 extract with the optimal extraction conditions was 62%.

The data regarding supercritical fluid extraction of lycopene could be useful for improving the value of tomato by-products and could serve as a basis for the creation of technology to isolate high-value biologically active components, thus improving the efficiency of industrial processing of tomatoes.

The response surface model analysis

As mentioned earlier, RSM using CCD was used to assess the influence of SC-CO_2 extraction parameters on the yields of non-polar fraction and *cis*-lycopene isomers. The following second-order polynomial model, which denotes an empirical relationship between the dependent variables and the independent test variables (*T, P, t*) was used:

$$Y_{oleor}\ (oleoresin\ yield, g/kg) = 198.82 + 45.40 \cdot T + 26.99 \cdot P + 9.94 \cdot t + 15.25 \cdot T \cdot P + 2.03 \cdot T \cdot t - 1.8 \cdot P \cdot t - 7.64 \cdot T^2 - 15.91 \cdot P^2 - 4.57 \cdot t^2 \quad (4.1)$$

$$Y_{isom}\ \left(cis\text{–}lycopene\ yield, \frac{g}{kg}\right) = 71.50 - 11.83 \cdot T + 4.86 \cdot P + 2.14 \cdot t + 2.84 \cdot T \cdot P + 0.094 \cdot T \cdot t + 0.049 \cdot P \cdot t - 11.17 \cdot T^2 + 1.96 \cdot P^2 + 0.005 \cdot t^2 \quad (4.2)$$

where Y_{oleor} is the response function of oleoresin yield (g/kg) and Y_{isom} is the response function of *cis*-lycopene isomer yield (g/kg).

The predicted values of non-polar fraction and *cis*-lycopene isomer yields were calculated using a second-order polynomial equation (3.2) and compared with the experimental values in Fig. 4.6. The adequacy of the model was evaluated with the total determination coefficient (R^2) values of 0.98 and 0.96, respectively, to Y_{oleor} and Y_{isom} yields. The determination coefficient indicates a reasonable fit to the experimental data. The adjusted coefficient of determination (R^2_{adj}) of 0.96 and 0.94 is in agreement with the predicted coefficient of determination (R^2_{pred}) of 0.93 and 0.90. The R^2_{adj} measures the amount of variation of the mean explained by the model.

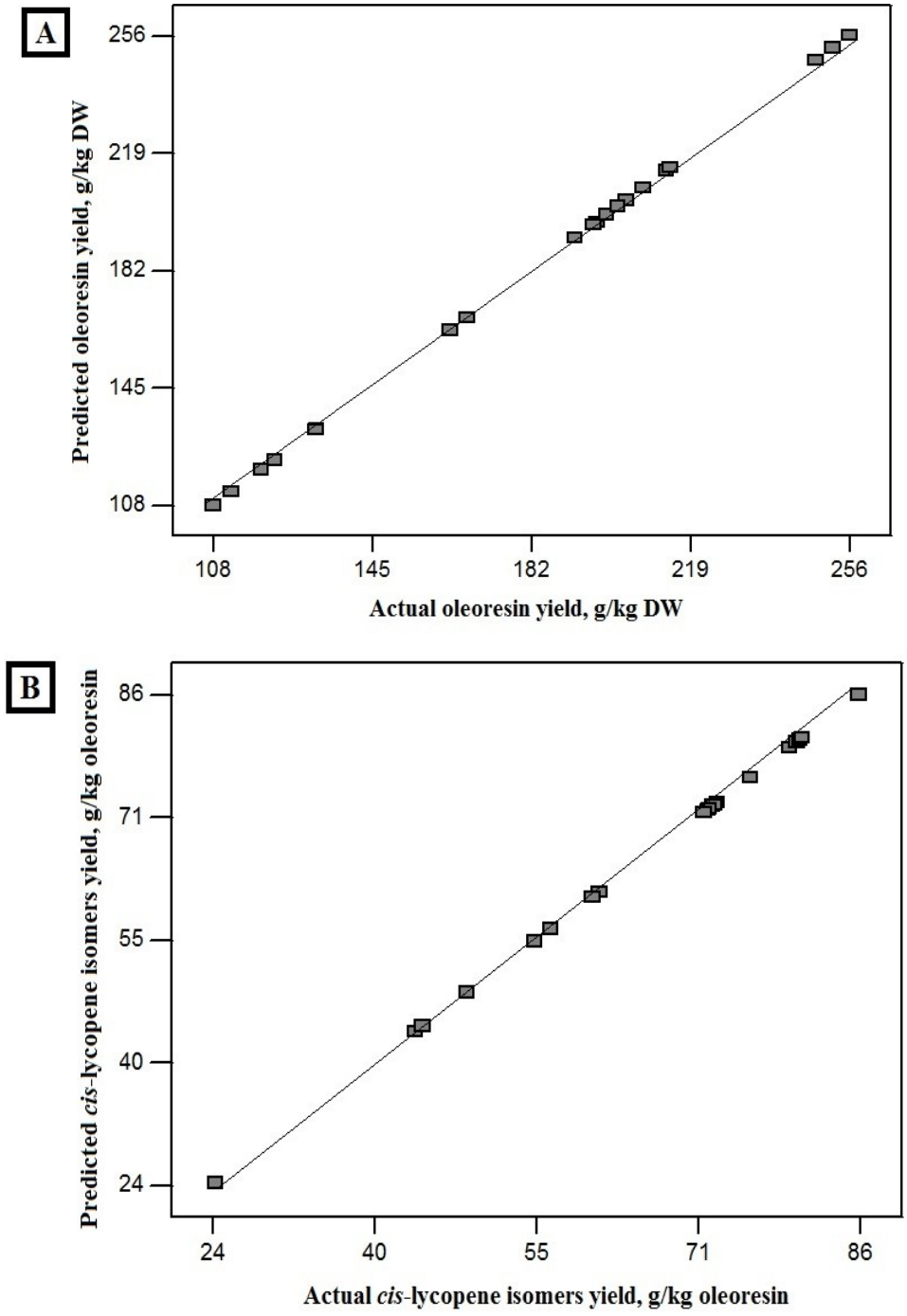


Fig. 4.6. A comparison of predicted values versus actual values for the oleoresin yield (A) and *cis*-lycopene yield in oleoresin (B) of tomato by-product

The statistical significance of the model was checked by *F*-test and *p*-value using Analysis of variance (ANOVA). The coefficients of the regression model estimated using regression analysis are given in Table 4.6 and in Table 4.7.

Table 4.6. Analysis of variance of the response Y_{oleo} surface quadratic model

Source	Sum of squares	df	Mean squares	*F*-ratio	*p*-Value
Model	41023.27	9	4558.14	27.64	<0.0001 significant
T	28154.30	1	28154.30	170.75	<0.0001
P	7760.85	1	7760.85	47.07	<0.0001
t	1348.49	1	1348.49	8.18	0.0170
TP	1861.11	1	1861.11	11.29	0.0072
Tt	32.97	1	32.97	0.20	0.6643
Pt	26.06	1	26.06	0.16	0.6993
T^2	848.45	1	848.45	5.15	0.0467
P^2	2290.86	1	2290.86	13.89	0.0039
t^2	303.31	1	303.31	1.84	0.2048
Residual	1648.87	10	164.89		
Lack of fit	1550.89	5	310.18	15.83	0.0044 significant
Pure error	97.98	5	19.60		
Cor total	42672.14	19			

In general, the analysis of the quadratic regression model showed that the model was significant ($p< 0.0001$ and $p<0.0005$, Y_{oleor} and Y_{isom}, respectively) (Table 4.6 and Table 4.7). The calculated *F*-values of the model were 27.64 and 10.48, which indicates that the model was significant. An adequate precision of 18.46 and 13.64 and the ratio greater than 4 is desirable, which indicates an adequate signal, therefore the model can be used to navigate the design space. An adequate of precision measures the signal-to-noise ratio, which compares the range of predicted values at the design points to the average prediction error.

The results reveal which parameters are significant and the most important in the model of Y_{oleo} response. The *p*-values of less than 0.05 indicate that the model terms are significant. In fact, there are six significant model factors – *T, P, t, TP,* T^2 and P^2. The model shows that the factor with the largest effect on the yield of oleoresin is extraction temperature (*T*) ($p<0.0001$), extraction pressure (*P*) ($p<0.0001$) and extraction time ($p<0.05$). The interaction between extraction temperature and pressure (*TP*) has the highest effect on the yield of oleoresin and is significant ($p<0.01$). The second-order terms of extraction time (T^2) and pressure (P^2) are also significant ($p<0.05$).

Therefore, there are only three significant model factors – *T, P* and T^2 (for the response of the yield of *cis*-lycopene isomer in oleoresin). The model shows that the factor with the largest effect on oleoresin yield was extraction temperature (*T*) (p<0.0001), followed extraction pressure (*P*) (p<0.05). The second-order term of extraction temperature (T^2) is significant with p<0.0001 (Table 4.7).

Table 4.7. Analysis of variance of the response Y_{isom} surface quadratic model

Source	Sum of squares	df	Mean squares	*F*-ratio	*p*-Value
Model	4306.9	9	478.6	27.64	0.0005 significant
T	1910.2	1	1910.2	170.75	<0.0001
P	323.2	1	323.2	47.07	0.0239
t	62.6	1	62.6	8.18	0.2688
TP	64.7	1	64.7	11.29	0.2614
Tt	0.07	1	0.07	0.20	0.9695
Pt	0.02	1	0.02	0.16	0.9841
T^2	1798.9	1	1798.9	5.15	c
P^2	55.3	1	55.3	13.89	0.2970
t^2	0.03	1	0.03	1.84	0.9977
Residual	456.7	10	45.7		
Lack of fit	455.6	5	91.1	15.83	<0.0001 significant
Pure error	1.09	5	0.22		
Cor total	4763.63	19			

In conclusion, the central composite design model is adequate for optimizing the SC-CO_2 extraction of oleoresin from tomato by-products and the yield of *cis*-lycopene isomers. The SC-CO_2 extraction parameters could be modified to increase the concentration of *cis*-lycopene isomers in the final product.

Higher temperature promotes the solubility of the solute and increases the yield by a high mass transfer of solute in the matrix and/or from the matrix to the solvent. At the same time, according to Reverchon, De Marco (2006), an important parameter in the SC-CO_2 extraction of oleoresins is the extraction pressure. The pressure affects the selectivity and solvent power of the supercritical fluid, which, in turn, determines the yield of the target compounds.

4.3. Stability and isomerisation of lycopene in oil-based model system during accelerated shelf-life storage

A comparison of literature data regarding the extension of carotenoid degradation in different food is difficult to compare. Due to the fact that different temperature and storage time conditions, processing and storage conditions are often

partially described, only total lycopene retention in food was described. There is a number of factors that influence the stability of biologically active constituents in tomatoes and tomato products. Although there are some data concerning the stability of carotenoids during tomato processing (George et al., 2011; Vallverdú-Queralt et al., 2015), there is still a lack of knowledge about the combined effect of different technological factors on the stability of individual carotenoid isomers in foods during long-term storage.

The data in literature generally agree that carotenoids are quite stable in fresh tomato matrices, but during thermal treatments, UV exposure or when carotenoids are dissolved in organic solvent, degradation and isomerisation can occur rapidly. Kinetic studies for biologically active components are capable of determining parameters, such as reaction order and constant rates, being equally important to establish the impact on the storage conditions for these components (Zepka et al., 2009). Consumers increasingly demand products of high quality (taste, appearance, texture, flavour) whilst keeping their nutritional value. For this purpose, determining the properties of food products during their shelf-life is very important for research and the food industry. The definition of shelf-life provides information regarding the time during which the product appropriately retains its quality (Ganje et al., 2016). This prediction could be performed by measuring the quality attributes through accelerated shelf-life testing (ASLT) under extreme conditions (Shao et al., 2015). However, ASLT could be beneficial for specifying the effects of different storage temperatures on the quality properties of food products in cases where the environmental conditions exceed the limits. Pedro and Ferreira (2006) used ASLT as a novel approach for determining the shelf-life of commercial concentrated tomato products and they reported zero- and first-order kinetic reactions for the quality factors of the product.

The effects of thermal- and light-irradiation processing on lycopene stability in an oil-based food model system have not yet been completely investigated. The stability of lycopene during heating and illumination has been studied but the results are controversial. Only a few studies have described the kinetics of *cis*-lycopene formation during the processing of tomatoes to tomatoes products (Ax et al., Shi et al., 2003; Colle et al., 2010).

The objectives of this study are to compare the effect of thermal and light treatments on lycopene isomerisation and stability in an oil-based food model system using accelerated shelf-life testing.

Lycopene isomerisation during temperature and illumination in an oil-based model system in accelerated shelf-life storage conditions was investigated. The control samples were samples at day 0. The all-*trans*-lycopene concentration of the control sample (at day 0) was equated to 100 %, and the concentration of *cis*-lycopene isomers was equated to 0%. In our study, the stability of lycopene isomers in the extract was investigated. The change of all-*trans*-lycopene and total *cis*-lycopene isomers over 200 days of storage is shown in Figures 4.7 and 4.8, respectively. The comparison of all treatments, namely, FT dark, AT light, AT dark, TT dark and TT UV with control shows that all-*trans*-lycopene decreases ($p < 0.05$). The all-*trans*-lycopene content decreases significantly ($p < 0.05$) in the TT dark and

TT UV conditions in comparison with other treatments. The amount of *trans*-lycopene changes from 100% to 51.70% at FT dark, to 0% at AT light, to 38.29% at AT dark, to 31.54% at TT dark and to 0% at TT UV conditions. Therefore, *trans*-lycopene in the AT light and TT UV samples after 200 days were not detected.

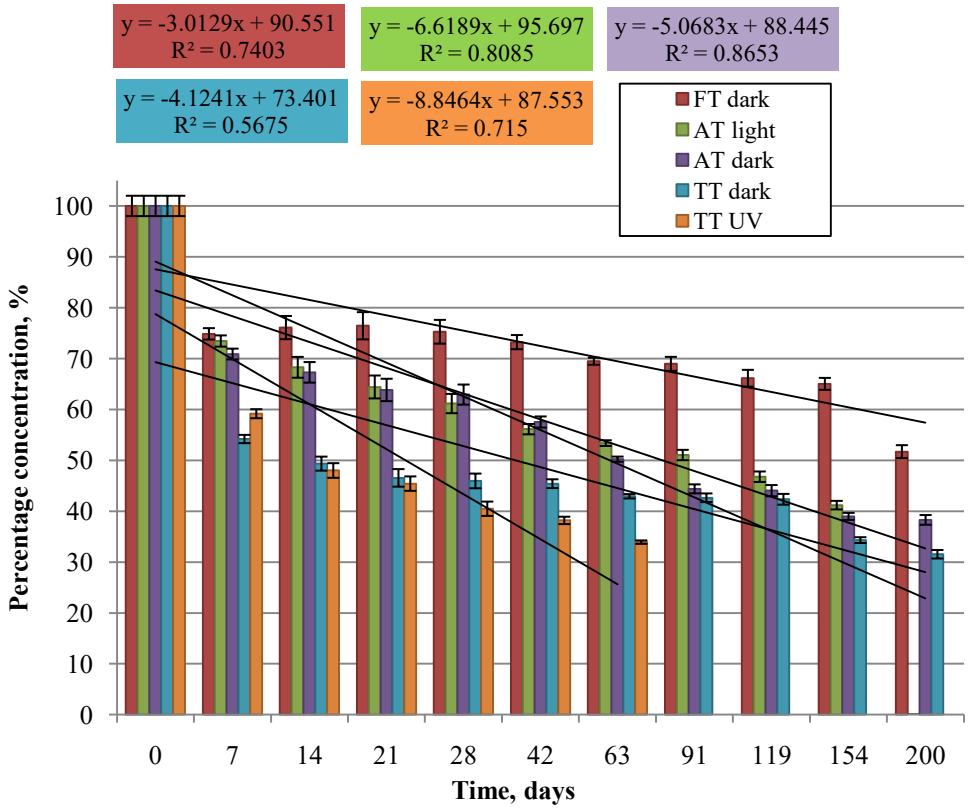


Fig. 4.7. Change of all-*trans*-lycopene during accelerated shelf-life testing impact over 200 days in an oil-based model system

Trans-lycopene degradation in the AT light and AT dark exhibits linear decline patterns with a correlation coefficient (R^2) greater than 0.8 for the plot of the concentration of all-*trans*-lycopene versus time. However, this linear characteristic fits only for the FT dark and TT UV samples with R^2 values ranging from 0.74 to 0.72, respectively. The linear characteristic does not fit well for the TT dark samples, with R^2 values of only 0.57, because the samples in FT dark treatment are the most stable in low temperature (1 ± 1°C) and absence of light (0 Lux). The results show that higher temperature directly correlates with the decreasing amount of *trans*-lycopene isomer. The decreased of amount of *trans*-lycopene may have been caused by isomerisation, resulting from additional energy (temperature and/or light) input, which led to unstable, energy-rich situations (Shi and Le Maguer, 2000).

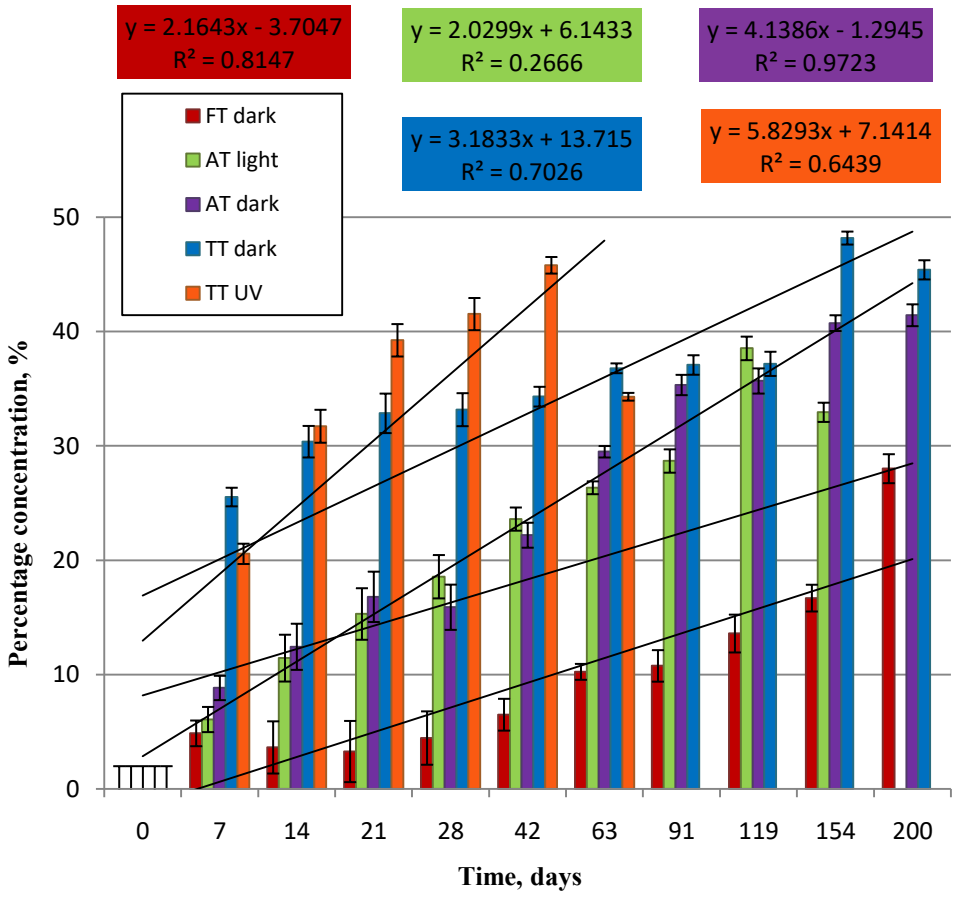


Fig. 4.8. Change of total-*cis*-lycopene during accelerated shelf-life testing impact over 200 days in an oil-based model system

According to the results of this study, the amount of total-*cis*-lycopene isomers increased in FT dark, AT dark and TT dark over 200 days. Excepting AT light and TT UV sample where the degradation process is stronger than isomerisation after 154 and 63 days, respectively (Fig. 4.8). The main change (more than 45%) of the total-*cis*-lycopene isomers in the oil-based model system was due to the influence of 37°C temperature in the TT dark samples. The amount of total-*cis*-lycopene isomers indicates that the *trans*-lycopene isomerizes to *cis*-isomers during the temperature

impacts: 28.0°C and 41.4°C for the FT dark and AT dark samples, respectively, after 200 days.

The total-*cis*-lycopene isomers means the sum of lycopene isomers identified (*5-cis*, *7-cis, 9-cis, 13-cis* and *15-cis*) and not identified (Table 4.8).

Table 4.8. The content of different *cis*-lycopene isomers in an oil-based food model system in accelerated shelf-life testing over 200 days

Storage time, days	Concentration of *cis*-lycopene isomers, µg/mL					
	total *cis*-	*5-cis*	7-cis	*9-cis*	13-cis	*15-cis*
0	–	–	–	–	–	–
			FT dark			
7	3.69±0.013f	3.71±0.025	0.16±0.032b	0.18±0.012a	0.00±0.002	0.28±0.037g
14	3.85±0.020f	3.13±0.014	0.19±0.012b	0.18±0.009a	1.44±0.026c	0.10±0.024c
21	4.72±0.015g	3.35±0.032	0.37±0.032c	0.26±0.010c	1.04±0.028a	0.18±0.003a
28	5.41±0.013d	3.42±0.021a	0.32±0.013a	0.30±0.026e	1.05±0.031a	0.18±0.008a
42	5.63±0.009a	2.84±0.012	0.25±0.032d	0.21±0.015b	0.96±0.018b	0.14±0.034b
63	5.64±0.016a	3.40±0.009	0.31±0.024a	0.20±0.030b	1.40±0.022c	0.19±0.012a
91	5.65±0.030a	4.29±0.019	0.23±0.018d	0.36±0.016f	1.03±0.004b	0.18±0.017a
119	5.77±0.029b	3.54±0.022	0.32±0.022a	0.23±0.032b	0.93±0.015b	0.14±0.006b
154	5.94±0.032c	1.25±0.017	0.15±0.039b	0.12±0.032d	0.78±0.029d	0.20±0.024d
200	6.53±0.021e	0.07±0.032	0.05±0.031e	0.05±0.032g	0.43±0.030e	0.05±0.022f
			AT light			
7	6.92±0.029a	3.89±0.030a	0.23±0.011c	0.48±0.015c	0.83±0.018c	0.13±0.008a
14	6.48±0.036b	4.45±0.018b	0.16±0.007b	0.64±0.013b	0.85±0.022c	0.14±0.011a
21	7.13±0.028c	4.39±0.042b	0.12±0.008a	0.19±0.018d	1.93±0.042a	0.23±0.032b
28	7.46±0.031c	4.77±0.027c	0.29±0.013d	1.00±0.025a	0.70±0.032d	0.14±0.014a
42	5.73±0.032d	3.86±0.034a	0.19±0.009c	0.63±0.048b	0.53±0.022f	0.10±0.032
63	6.99±0.023a	3.92±0.022a	0.16±0.007b	0.90±0.018a	1.28±0.029b	0.22±0.019b
91	4.70±0.019f	2.50±0.012d	0.06±0.002e	0.46±0.012c	0.66±0.035d	0.12±0.032a
119	1.93±0.012g	1.30±0.018f	0.02±0.005e	0.19±0.018d	0.15±0.009g	0.06±0.032c
154	0.23±0.009h	Nd.	Nd.	Nd.	Nd.	Nd.
200	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
			AT dark			
7	3.92±0.034c	3.67±0.024b	0.18±0.003a	0.24±0.004ab	2.17±0.014g	0.28±0.003c
14	4.31±0.029c	4.46±0.036b	0.19±0.004a	0.27±0.002b	1.62±0.024f	0.23±0.006c
21	7.74±0.044b	4.99±0.030a	0.27±0.009c	0.99±0.005	0.75±0.004c	0.14±0.004a
28	8.68±0.038a,b	6.23±0.040c	0.25±0.005c	0.29±0.006b	0.97±0.005d	0.17±0.002a
42	7.52±0.041b	5.40±0.035a	0.27±0.004c	0.46±0.004c	0.90±0.006d	0.12±0.003a
63	9.52±0.033a	6.31±0.032c	0.31±0.010d	0.51±0.003d	1.74±0.004f	0.24±0.004c
91	10.63±0.025a	6.68±0.031c	0.53±0.004f	0.66±0.005e	2.16±0.008g	0.27±0.002c
119	6.86±0.043b	4.69±0.028a	0.40±0.009e	0.50±0.004d	1.06±0.009e	0.13±0.001a
154	2.25±0.034e	1.32±0.023d	0.11±0.004b	0.19±0.003a	0.16±0.004a	0.17±0.004b
200	2.75±0.031c,e	1.62±0.019d	0.17±0.003a	0.29±0.005b	0.42±0.002b	0.05±0.001d

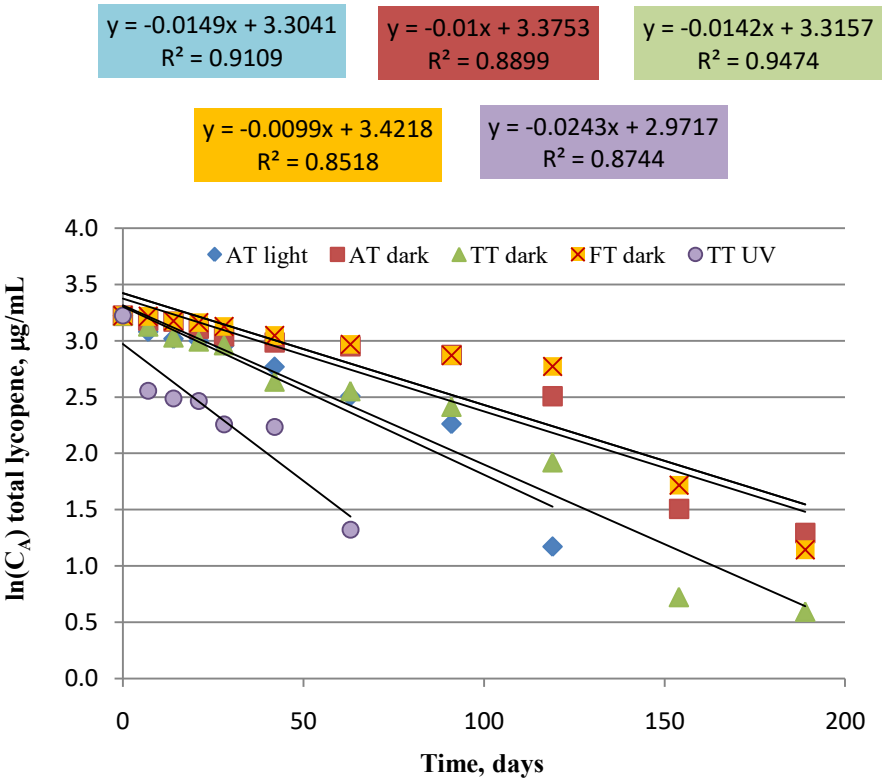
Storage time, days	Concentration of *cis*-lycopene isomers, µg/mL					
	total *cis*-	*5-cis*	7-cis	*9-cis*	13-cis	*15-cis*
0	–	–	–	–	–	–
			TT dark			
7	10.44±0.23a	6.23±0.031c	0.30±0.011b	0.64±0.008b	2.26±0.030f	0.34±0.003d
14	10.99±0.41a	7.29±0.020c	0.52±0.013c	1.07±0.009c	1.31±0.021e	0.21±0.001c
21	9.77±0.33a	6.85±0.031c	0.47±0.009c	1.14±0.013c	0.77±0.021d	0.14±0.002b
28	10.66±0.21a	7.02±0.023c	0.80±0.005e	1.38±0.018e	0.81±0.009d	0.17±0.005
42	12.83±0.28a	4.66±0.019b	0.47±0.011c	0.85±0.014b	0.55±0.003c	0.11±0.001a
63	7.97±0.053b	4.73±0.043b	0.67±0.012d	1.37±0.023d	0.73±0.013d	0.14±0.003b
91	6.41±0.041b	3.97±0.025a	0.49±0.013c	1.21±0.043d	0.47±0.018c	0.11±0.002a
119	3.91±0.038c	2.44±0.026d	0.26±0.009b	0.65±0.013b	0.08±0.002a	0.08±0.001a
154	1.41±0.019d	0.56±0.005e	0.12±0.005a	0.34±0.010a	0.22±0.004b	0.07±0.001a
200	1.19±0.013d	0.50±0.003e	0.09±0.002a	0.24±0.009a	0.18±0.003b	0.09±0.001a
			TT UV			
7	7.81±0.039b	3.12±0.036c	0.04±0.001c	0.54±0.013a	0.10±0.005b	0.12±0.006a
14	9.70±0.12ab	4.48±0.041b	0.23±0.003a	0.82±0.009b	0.77±0.006b	0.14±0.008ab
21	10.16±0.090a	5.09±0.023b	0.20±0.004a	0.72±0.007b	0.77±0.007b	0.13±0.009a
28	8.21±0.043b	3.49±0.033a	0.18±0.002b	0.65±0.011a	0.57±0.002a	0.12±0.003a
42	6.78±0.030c	3.98±0.031a	0.25±0.004a	0.59±0.008a	0.69±0.003a	0.11±0.001a
63	5.77±0.021c	3.94±0.023a	0.23±002a	0.49±0.009a	0.90±0.011c	0.15±0.005ab
91	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
119	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
154	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.
200	Nd.	Nd.	Nd.	Nd.	Nd.	Nd.

Values of means bearing different letters in the same column are significantly different ($p<0.05$); Nd. – not detected.

Our investigation shows that the *5-cis*-isomer changes distinctively during lycopene storage compared to the other lycopene isomers (*7-cis, 9-cis, 13-cis* and *15-cis*) (Table 4.8). The *5-cis*-isomer of lycopene indicates the main isomerisation process because this isomer was dominant in all the samples during the 200 days of storage. *5-cis*-lycopene isomer in the FT dark sample was almost stable over 200 days, which can be caused by low temperature (1 ± 1°C) and no illumination (0 Lux). The latter conditions slow down the isomerisation and degradation processes for lycopene. When comparing the 20 ± 1°C temperature (AT light and AT dark samples) and the 37ºC temperature (TT dark and TT UV samples), the results show that the temperature impact causes the isomerisation and degradation of *cis*-lycopene isomers after long-term storage.

It is difficult to compare our results with recent studies, because little is known about the stability of lycopene in an oil-based food model system. Zhang and colleagues (2012) studied thermally-induced geometrical isomerisation of lycopene dissolved and isomerised in ethyl acetate at 78 °C temperature. The temperatures used in Shi and Le Maguer (2000) and Chen et al. (2009) studies varied from 80°C to 180°C and they studied the stability of lycopene in tomato products.

The degradation rate constant (k) of the total lycopene content in the oil-based model system under accelerated shelf-life storage conditions was fitted to a first-order kinetic reaction model, as shown in Figure 4.9. The kinetic degradation rate of total lycopene in the oil-based model system shows a good-fit kinetic model with a linear correlation (R^2) range of 0.89–0.95 for the AT light, AT dark and TT dark samples, in contrast to the FT dark and TT UV samples with R^2 values of 0.85 and 0.87, respectively. The kinetic degradation rate constant (k in day^{-1}) resulted in total lycopene loss rates (in μg mL^{-1} in oil) of 0.009, 0.015, 0.010, 0.014 and 0.024 during the temperature and light impact in the FT dark, AT light, AT dark, TT dark and TT UV samples, respectively, in the oil-based model system. The largest kinetic constant (k = 0.0243) of the total lycopene degradation was for the TT UV sample (Figure 4.9).



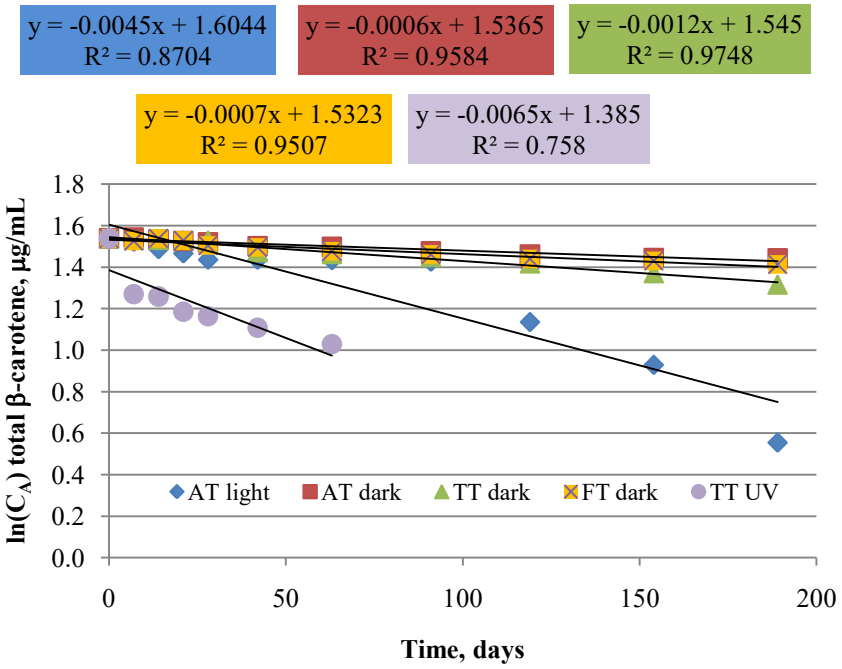
Note: The results are from the means of three HPLC analyses of three extracts; C_A – the total amount of lycopene after storage

Fig. 4.9. A plot of ln (concentration) *vs* time data for a first-order reaction of total lycopene

According to the results of this study, the reasons for the differences in kinetic constants are unclear. The main explanation could be related to the fact that there was a slight but not significant change in the total lycopene content in the FT dark sample, so the linear relationship did not exist. In contrast, highly significant changes were found in the TT UV sample because UV irradiation could have led to

the degradation of total lycopene content.

The kinetic data of lycopene stability could be a complex process, and the data of kinetic study could depend on other compounds in the same food-based study. The high R^2 value (0.95–0.975) of total β-carotene in the FT dark, TT dark and AT dark samples indicates that the kinetics of the reaction proceeded as a first-order reaction (Figure 4.10), and the slope provided the first-order rate constant. Because the concentration of the β-carotene is decreased, the slope was negative. Therefore, we used the absolute value to obtain the result for the rate constant. The R^2 value for a linear regression between the concentration and time data shows a good correlation for the TT UV and AT light samples with the values of 0.76 and 0.87, respectively, indicating that the kinetics of this reaction was also first-order.



Note: The results are from the means of three HPLC analyses of three extracts; C_A – the total amount of β-carotene after storage

Fig. 4.10. A plot of ln (concentration) *vs* time data for a first-order reaction of total β-carotene

Therefore, the natural logarithms of the concentration values were calculated, and the slope was determined. The kinetic degradation rate constant (k in day^{-1}) indicated that the rate of total β-carotene loss (in µg mL^{-1} in oil) during the temperature and light impact was 0.0007, 0.0045, 0.0006, 0.0012 and 0.0065 in the oil-based food model for the FT dark, AT light, AT dark, TT dark and TT UV samples, respectively. The largest kinetic constant (k = 0.0065) for the total β-carotene degradation was found for the TT UV sample.

Colour change of lycopene oil-based food system

The changes in colour values (a* to b* ratio; hue angle, h°; chroma C and colour difference (ΔE) of the control and samples over the course of 200 days of storage at 1, 20 and 37 ± 1 °C storage temperatures) are shown in Table 4.9. In comparison to the control sample at day 0, the colour of all of the processed samples (AT light, AT dark, TT dark and FT dark) changed as a function of storage temperature, illumination and time. The lycopene after 63 days in TT UV sample was not detected, therefore the TT UV sample was eliminated from the results. The distinctions in colour between different samples were noticed with untrained human eye after 200 days of storage (Supplement 5).

A slight difference of a* to b* ratio (a* to b* ratio indicates the colour changes from red to yellow) in the sample set is found after 200 days, excepting TT dark samples. In comparison with other samples, the a* to b* ratio differences of the TT dark sample were significant (from control sample; 0.64 to 0.10 after 200 days), it means that the colour changed to yellow. The hue angle varied from 56.2° (AT light) to 59.1° (TT UV) (90° would mean that the sample is yellow, and 0° indicates a completely red sample) after 200 days. The results suggest that all samples have a reddish-yellow colour after 200 days compared to the colour at day 0. The chroma value indicates the degree of colour saturation and is proportional to the strength of the colour. There are no significant differences in the chroma value between day 0 and 200 in the FT dark and AT dark sample (34.6 and 34.9, respectively) (Table 4.9).

Table 4.9. The changes in colour values (a* to b* ratio; chroma, C; hue angle, h°, colour difference, ΔE) of the control and samples over the course of 200 days of storage at 1, 20, and 37 ± 1°C temperatures

Sample name	Colour parameters							
	a* to b* ratio		C		h°		ΔE	
Control sample	0.64	±0.025	35.13	±1.40	57.46	±2.18	–	
After 200 days								
AT light	0.40	±0.028 a	36.4	±1.25 ab	56.23	±2.06 a	7.29	±0.013 a
AT dark	0.55	±0.020 a	34.9	±1.32 a	57.15	±2.29 a	2.38	±0.011 b
TT dark	0.10	±0.026 ab	28.2	±1.16 b	59.10	±1.48 b	21.7	±0.054 c
FT dark	0.65	±0.030 a	34.6	±1.39 a	56.15	±2.17 a	0.81	±0.028 c
TT UV	–		–		–		–	

Notes: The numbers are means followed by standard deviations (n = 3). Means within a column with different superscript letters are significantly different ($p \leq 0.05$). AT light – ambient temperature at 20 ± 1°C in natural light with different day and night illumination; AT dark – ambient temperature at 20 ± 1°C in absence of light; TT dark – thermostatically controlled temperature at 37 ± 1°C in absence of light; FT dark – fridge temperature at 1 ± 1°C in absence of light; TT UV – thermostatically controlled temperature at 37 ± 1°C in UV irradiation.

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.

Our study indicates that the chroma value of the TT UV sample increased until day 21 and then decreased until the end of storage. The total colour difference (ΔE) is more than 3 in the TT UV sample after 200 days. A value of $\Delta E \approx 2$ represents a noticeable colour difference, and $\Delta E > 3$, for many products is unacceptable, according to consumers. Among the treatments, the FT dark, AT light, AT dark, and TT dark samples present better colour retention, and the TT UV samples show the most colour changes. The FT dark, AT dark, AT light and TT dark samples did not show any colour loss (Table 4.9).

4.4. A study of the influence of fermentation with lactic acid bacteria on the recovery of carotenoids from tomatoes and tomato by-products

Many biochemical changes occur during fermentation, leading to an altered ratio of nutritive and anti-nutritive components and, consequently, affect the properties of the products, such as bioactivity and digestibility (Hur et al., 2014). Recently, this bioprocess has been applied to the production and extraction of bioactive compounds in the food, chemical and pharmaceutical industries (Torino et al., 2013). According to Torino et al. (2013), fermentation has been applied to the recovery of different biologically active substances from plant material. Although the fermentation process involves commercial enzymes, there is the possibility to use LAB for the fermentation processes. LAB, as mentioned before, are recognized as safe and traditionally used to ferment raw materials of vegetable origin in the industrial production. These processes have been thoroughly investigated not only because of their physiological significance, but also because of their technological importance in the development of texture and flavour (Caplice and Fitzgerald, 1999; Adams and Mitchell, 2002; Bourdichon et al., 2012).

Based on these studies, it is interesting to assess the effect of fermentation with lactic acid bacteria (*L. sakei* – *Lactobacillus sakei* KTU05-6, *P. acidilactici* – *Pediococcus acidilactici* KTU05-7, *P. pentosaceus* – *Pediococcus pentosaceus* KTU05-8) on the recovery of carotenoids from tomatoes and tomato by-products and to evaluate the possible lycopene isomerisation during fermentation of different tomato varieties and the powder of tomato by-products.

Characterisation of fermented tomato by-product powder

As reported in literature, the behaviour of different LAB depends on the composition of substrate, since in different substrates bacteria are able to produce different metabolites or increase the biomass (Kedia, Vazquez and Pandiella, 2008). The tomato by-product powder (TP) (recommended as GRAS) was fermented with *L. sakei* and *P. pentosaceus.*

The results of bacterial growth during the fermentation of tomato by-product powder are presented in Fig. 4.11. The fermentation of sugars leads to the formation of lactic acid as the major end product. The study shows that TP fermentation time has significant ($p \leq 0.05$) influence on bacterial growth rate (Fig. 4.11). The most intensive cell growth can be noticed of the *L. sakei* strain (7.53 $\log_{10}$ CFU/g) followed by the *P. pentosaceus* strain (6.35 $\log_{10}$ CFU/g) during 24 h of fermentation. Spontaneous fermentation of TP induces lower cell growth compared to fermentation with pure LAB. The TP medium seems to be most suitable for *L. sakei*; the viable cell count of the bacteria reached 8.15 $\log_{10}$ CFU/g after 48 h of fermentation.

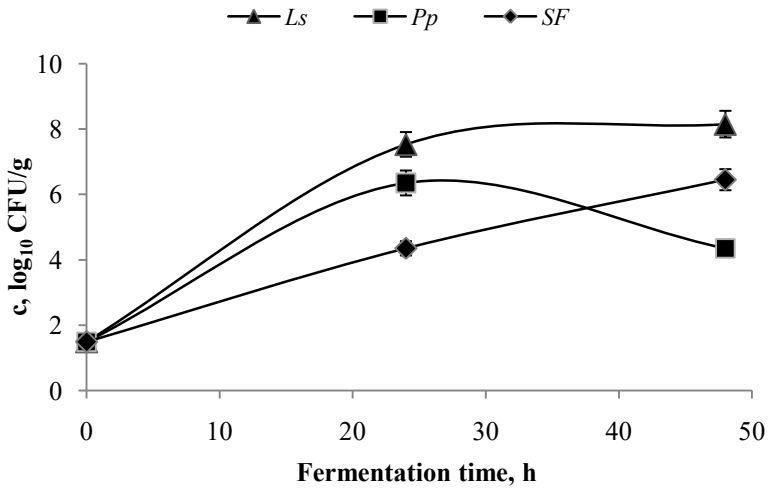


Fig. 4.11. The viable cell counts of tested LAB *vs.* fermentation time in tomato powder (TP) fermented with different LAB: *Pp – P. pentosaceus*, *Ls – L. sakei, SF* – spontaneous fermentation

A significant ($p \leq 0.05$) reduction in viable cells can be found in the TP samples fermented with *P. pentosaceus* after 48 h of fermentation. In contrast, the spontaneous fermentation of TP shows an increase of bacteria cell counts during longer fermentation (Fig. 4.11). For health benefits, probiotic bacteria must be viable and available at a high content, typically about 6 $\log_{10}$ CFU/g of product (Shah, 2001).

The results of pH change during tomato powder fermentation are presented in Fig. 4.12. The formation of organic acids is noticed in the TP samples. Significant differences are not found in the pH value. The pH of the medium decreases from 4.1 to 4.15 (Fig. 4.12).

In selecting probiotic bacteria for industrial manufacturing processes, it is important to consider their safety, functional and technological characteristics. Functional properties include the viability of cells, their stability within a food matrix, strain characteristics and fermentation technology (Georgieva et al., 2009; Rathore, Salmeron, Pandiella, 2012).

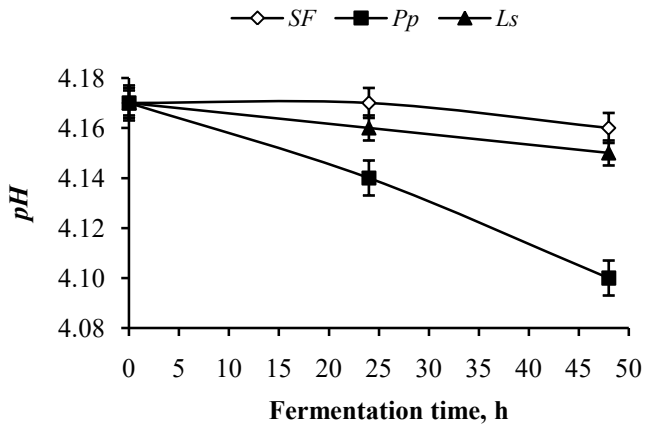


Fig. 4.12. The pH values *vs*. fermentation time in tomato powder (TP) fermented with different LAB: *Pp – P. pentosaceus*; *Ls – L. sakei*; *SF* – spontaneous fermentation

Our results support that the LAB counts compare to the value of 6.35–8.15 $\log_{10}$ CFU/g, which is considered to be the acceptable probiotic count for being functional.

The recovery of carotenoids from tomatoes and tomato by-products

The results of the fermentation with different lactic acid bacteria strains on the recovery of lycopene and β-carotene from tomatoes are presented in Fig. 4.13. The recovery of total carotenoids (on average of 6.83 mg/100 g DW) was measured in var. Cunero sample fermented with *P. pentosaceus* and in var. Ronaldo sample fermented with *L. sakei*. However, fermentation with lactic acid bacteria changed the recovery of total carotenoids by 37%, to compare with untreated samples (Fig. 4.13). The difference in results can be due to the chemical composition of tomatoes which depends on tomato varieties and different growing conditions. Also, as mentioned before, the behaviour of different LAB depends on the substrate composition.

Fermented tomato samples of Cunero variety lowers the recovery of β-carotene on average by 24.7% and increases the recovery of lycopene by 11.5% compared to the untreated tomatoes. On the other hand, the recovery β-carotene in all fermented tomato products of Ronaldo variety is generally higher (Fig. 4.13).

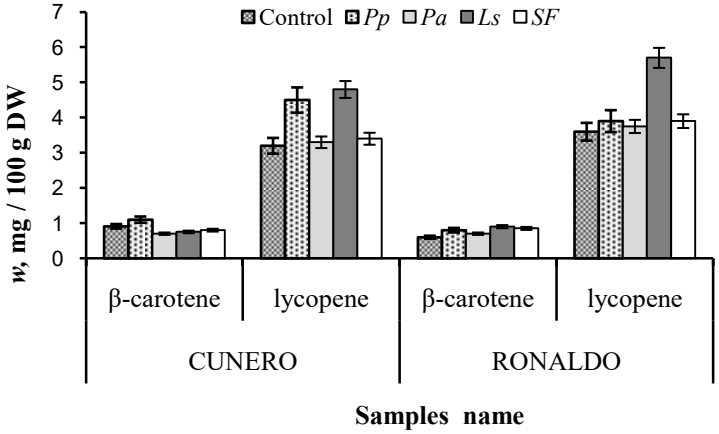


Fig. 4.13. Carotenoid contents in untreated and fermented with different LAB tomato samples. Samples: Control – untreated tomato by-products powder; tomato by-products powder fermented with: Pp – *P. pentosaceus;* Pa – *P. acidilactici MI807;* Ls – *L. sakei,* SF – spontaneously fermented

Based on the results, the effect of fermentation with lactic acid bacteria on the lycopene isomerisation during fermentation of different tomato varieties does not have a significant effect (Fig. 4.14).

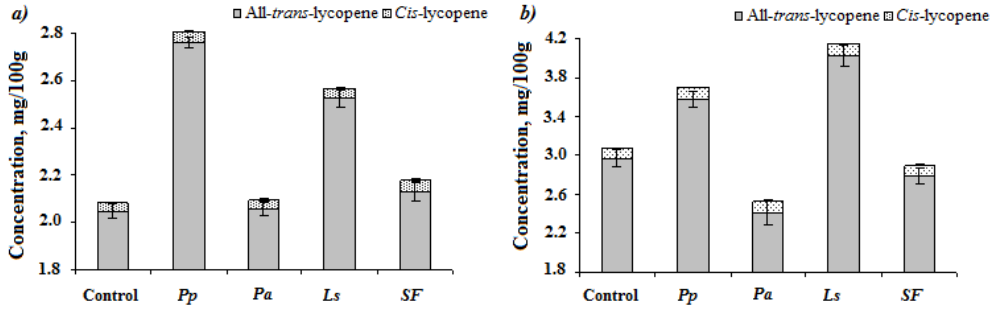


Fig. 4.14. Concentrations of all-*trans*- and *cis*-lycopene isomers in fermented tomato of var. Cunero (a) and var. Ronaldo (b) products. Samples: Control – unreated tomato by-products powder; tomato by-products powder fermented with: P.p. – *P. pentosaceus,* P.a. – *P. acidilactici,* L.s. – *L. sakei*; SF – spontaneous fermentation

The results of carotenoid recovery from tomato by-product powder (TP) and their colour measurements are presented in Fig. 4.15. Fermentation significantly ($p \leq 0.05$) recovers the contents of carotenoids and influences the colour characteristics of the tomato powder products (Fig. 4.15). Total carotenoid concentrations of up to 95.56 and 98.96 mg/100 g (DW) are measured in tomato samples fermented with *P. pentosaceus* and *L. sakei*, respectively. In addition, spontaneous fermentation (SP) recovers the carotenoids, but not effectively when compared with fermentation with selected LAB.

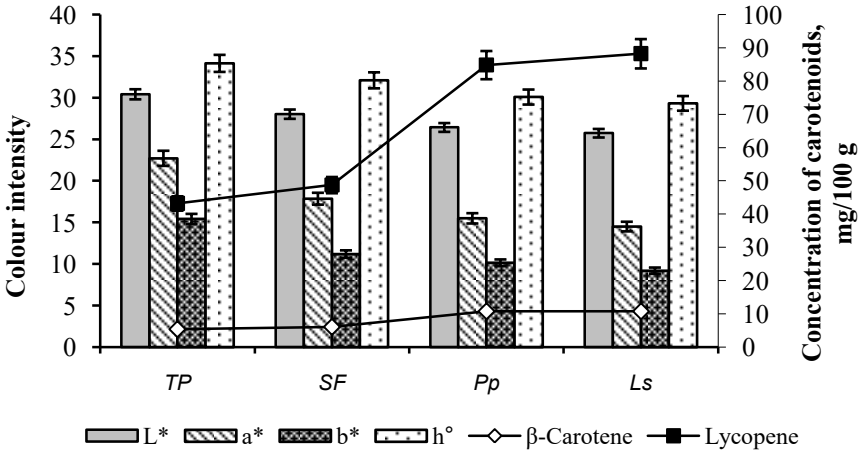


Fig. 4.15. Colour characteristics and carotenoid contents of non-treated and fermented tomato powder (TP). *SP* – spontaneously fermented, *Pp* – fermented with *P. pentosaceus*, *Ls* – fermented with *L. sakei*

Colour is an important quality attribute in the food and bioprocess industries that influences consumer choice and preference. The measurement of food product colour has been used as an indirect evaluation of other quality attributes, such as flavour and content of pigments, due to the simple and fast measurements and the good correlation with other physicochemical properties (Pathare et al., 2013).

A significant ($p \leq 0.05$) decrease in yellowness (b*) and redness (Fig. 4.15) with increased contents of β-carotene and lycopene in the TP after 48 hours of fermentation can be detected. In all TP samples, the colour tone (h°) shows mean values ranging from 29.3 to 34.1, indicating an orange colour. Thus, the samples with a lighter colour (higher L* values) tend to be more yellow-orange than the samples with a darker colour. However, the lacto-fermented samples show a colour tone (h°) lower by 7.3 % and 12.9 % compared with the SPF and TP samples, respectively (Fig. 4.15). A strong negative linear correlation is observed between β-carotene and lycopene contents and lightness (L*) (R^2=0.835 and R^2=0.846, respectively), and also between the colour tone (h°) and lycopene contents (R^2=0.904, $p \leq 0.001$). A strong positive linear relationship is observed between L* and colour tone (h°) (R^2 = 0.830, $p \leq 0.001$). The colour change of a tomato product is an indication of the fermentation conditions and is related to its pigment recovery from the tomato matrix.

The results of this study indicate that the pretreatment of feed material during different technological processing is very important. The recovery of carotenoids from the tomato by-products and tomatoes can be enhanced by the use of fermentation with LAB. There is the possibility to combine fermentation with LAB assisted extraction with SC-CO_2 in order to increase the yield of extracted fractions. This fact, together with the comparatively low-cost tomato by-products, lends strong support to the possible implementation of the process on an industrial scale.

The production of L- and D-lactic acid during lacto-fermentation of tomato by-products

This study analysed *P. pentosaceus*, *P. acidilactici*, *L. sakei* and spontaneous fermentation of tomato by-product powder. The results indicate that all analysed LAB produce the mixture of L- and D-lactic acid (Fig. 4.16). The highest levels of both forms are determined in tomatoes treated by spontaneous fermentation (7.18±0.03 and 7.67±0.11 mg/100 g, respectively). As it was reported by Hartman (1998) and Li and Cui (2010), the *La. amylophilus*, *L. bavaricus*, *L. casei*, *L. maltaromicus* and *L. salivarius* predominantly yield the L-isomer. Strains such as *L. delbrueckii*, *L. jensenii* or *L. acidophilus* yield the D-lactic acid or mixtures of both forms. Lactic acid bacteria, such as *L. pentosus*, *L. brevis* and *L. lactis* can ferment glucose to lactic acid by homolactic fermentation. Fermentation of rice with two strains of *L. delbrueckii* yielded the 3.23 and 5.04 mg/100 g of D-lactic acid (Lee, 2007).

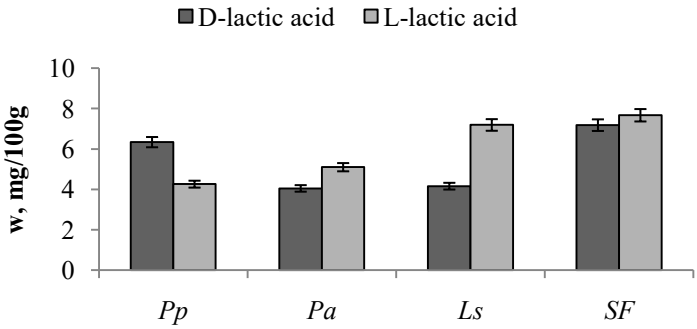


Fig. 4.16. Mass fraction of L- and D-lactic acid in fermented tomato products. Samples fermented with LAB: *Pp – P. pentosaceus*, *Pa – P. acidilactici*, *Ls – L. sakei*; *SF* – spontaneously fermented

Concentrations of D-lactic acid in fermented tomato products measured between 4.1±0.05 and 6.3±0.04 mg/100 g, and concentrations of L-lactic acid ranged from 4.3±0.04 to 7.2±0.08 mg/100 g (Fig. 4.16). The results of our study indicate that the use of *P. pentosaceus* allows reducing the content of D-lactic acid in tomato products by 11.8% compared to spontaneous fermentation (Fig.4.16). Fermentation with *P. acidilactici* and *L. sakei* reduces the content of the latter isomer at a higher level (on average by 40%).

In summary, the *P. pentosaceus* can produce D-rich lactic acid (L/D ratio 0.64), while the other strain of *L. sakei* produces L-rich lactic acid (L/D ratio 1.61). Fermentation with *P. acidilactici*, as well as spontaneous fermentation, produces almost an equal amount of both lactic acid isomers (L/D ratio 1.17 and 1.07, respectively).

After an evaluation of knowledge of the potential toxicity of D-lactic acid in terms of nutrition, we could report that tomatoes prepared using pure cultures of LAB in all cases are found to be safer than treated by spontaneous fermentation. The levels of D-lactic acid with pure LAB fermented tomato products are significantly

lower (p<0.05) than that of spontaneously fermented (Fig. 4.16). Based on the results, *L. sakei* could be selected as the L-lactic acid bacteria and could be recommended for safe fermentation of tomatoes and tomato by-products.

The improvement of the prototypical ready-to-cook minced meat with fermented tomato by-products

Several studies have reported the use of tomato products or lycopene in meat products. Candogan (Candogan, 2002) and Deda et al. (2007) reported on the impact of the addition of tomato paste or tomato peels on the quality and sensorial properties of beef patties and frankfurters. Calvo et al. (2008) used tomato peels for the enrichment of dry fermented sausages with lycopene. However, data were not found on the effect of fermented tomato products on the quality parameters of ready-to-cook minced meat products (RCMP).

The aim of this study is to evaluate the influence of fermented tomato by-product powder (TP) with bacteriocin-like inhibitory substances (BLIS) produced by *P. pentosaceus* and *L. sakei* on the quality and effect on the colour, carotenoid content and sensory characteristics of RCMP before and after thermal treatment (10 min in 100 °C water).

The results of the carotenoid analysis for the RCMP are presented in Table 4.10. The concentrations of lycopene and β-carotene in the RCMP increase from 0.48 to 10.32 mg/100 g and from 0.19 to 1.95 mg/100 g, respectively, according to the percentage of TP in the formulation (10 or 30 %) (Table 4.10). The addition of lacto-fermented TP provides a mean concentration of β-carotene and lycopene of 1.12 and 3.63 mg/100 g (10 % of TP), respectively, and 1.86 and 9.99 mg/100 g (30 % of TP), respectively. The RCMP with spontaneously fermented TP additives show concentrations of β-carotene and lycopene that are 43 and 75 % lower, respectively, when compared to the RCMP with lacto-fermented TP. The lowest concentrations of β-carotene and lycopene are measured in the RCMP samples with 10 and 30 % of non-fermented TP (0.19 and 0.69 mg/100 g, respectively).

Cooking the RCMP in water lowers the contents of β-carotene and lycopene as well as the total carotenoid contents (Fig. 4.18). Average carotenoid losses of up to 22–28 % and up to 30–41 % can be detected in RCMP samples with 10 and 30 % of fermented TP, respectively. As a consequence, the highest loss of total carotenoids is found in samples with 10 and 30 % of non-fermented TP (38 and 49 %, respectively) (Table 4.10).

Table 4.10. The effect of the addition of fermented tomato by-product powder on the carotenoid concentration (mg/100 g) in non-treated and thermally treated (10 min in 100 °C water) ready-to-cook minced meat

RCMP		β-Carotene	Lycopene	Total carotenoids
Non-treated				
10 % additive	Pp	1.01±0.07^{d}	3.67±0.21^{d}	4.68±0.13^{d}
	Ls	1.23±0.09^{d}	3.59±0.21^{d}	4.82±0.11^{c}
	SF	0.69±0.02^{c}	1.02±0.09^{b}	1.71±0.06^{b}
	TP	0.19±0.02^{a}	0.48±0.06^{a}	0.67±0.05^{a}
30 % additive	Pp	1.76±0.03^{e}	10.32±0.11^{e}	12.08±0.07^{e}
	Ls	1.95±0.08^{e}	9.66±0.17 ^{e}	11.61±0.14^{e}
	SF	0.97±0.02^{c}	2.13±0.15^{c}	3.1±0.09^{c}
	TP	0.25±0.01^{b}	1.46±0.10^{b}	1.71±0.06^{b}
Thermally treated				
10 % additive	Pp	0.76±0.06^{c}	2.62±0.20^{c}	3.38±0.14^{c}
	Ls	0.59±0.06^{b}	2.15±0.13^{c}	2.74±0.20^{c}
	SF	0.49±0.07^{b}	0.91±0.06^{b}	1.40±0.05^{b}
	TP	0.10±0.02^{a}	0.24±0.07^{a}	0.34±0.03^{a}
30 % additive	Pp	1.17±0.10^{d}	6.31±0.26^{d}	7.48±0.17^{d}
	Ls	1.47±0.08^{d}	7.32±0.14^{d}	8.79±0.09^{d}
	SF	0.74±0.04^{c}	1.75±0.09^{c}	2.49±0.07^{c}
	TP	0.15±0.04^{a}	0.93±0.05^{b}	1.08±0.05^{b}

Means in columns with different letters are significantly different ($p \leq 0.05$).
RCMP samples: Control – without additives, TP – with tomato powder, SF – with spontaneously fermented TP, Pp – with *P. pentosaceus* fermented TP, Ls – with *L. sakei* fermented TP

Our study shows that the addition of fermented TP significantly affects ($p \leq 0.05$) the colour characteristics of the RCMP (Table 4.11). The controls (without TP) have significantly higher ($p \leq 0.05$) values of lightness (L*) and the lowest ($p \leq 0.05$) values of redness (a*) and yellowness (b*). The addition of 10 and 30 % of fermented TP increases the redness by 70.2 and 77.8 % and yellowness by 1.8 and 1.9 %, respectively, of the RCMP, which are more attractive to consumers than the control samples (Table 4.11).

The content of lycopene in RCMP with lacto-fermented TP is higher than that reported by Domenech-Asensi et al. (2013) in sausage mortadella after the addition of tomato paste. The lycopene in our RCMP samples is expected to be more available, since it is from fermented tomato powder in which fermentation with LAB leads to higher recovery and perhaps the bioavailability of total carotenoids.

Table 4.11. The effect of the addition of fermented tomato by-products (TP) on colour characteristics of non-treated and thermally treated (10 min in 100 °C water) RCMP

RCMP		*L**	*a**	*b**	*h*°
Non-treated					
Control		58.38±0.48^{e}	3.93±0.18^{a}	15.33±0.78^{a}	81.18±0.54^{e}
10 % additive	Pp	50.64±0.19^{d}	12.90±0.72^{c}	26.90±0.74^{d}	65.99±0.93^{d}
	Ls	50.90±0.79^{d}	12.14±0.21^{c}	27.60±0.52^{de}	66.93±0.88^{d}
	SF	45.21±0.41^{a}	14.02±0.19^{d}	21.45±0.41^{c}	61.33±0.46^{b}
	TP	44.25±0.39^{a}	11.03±0.11^{b}	19.56±0.59^{b}	60.84±0.93^{b}
30 % additive	Pp	46.03±0.25^{a}	17.27±0.51^{e}	28.96±0.83^{f}	57.30±0.40^{a}
	Ls	45.63±0.63^{a}	18.08±0.61^{e}	28.50±0.48^{f}	56.77±0.68^{a}
	SF	47.23±0.28^{b}	15.23±0.34^{d}	25.36±0.39^{d}	62.39±0.45^{c}
	TP	46.27±0.52^{b}	13.25±0.16^{c}	21.55±0.57^{c}	61.45±0.41^{b}
Thermally treated					
Control		60.07±0.43^{e}	2.41±0.30^{a}	11.84±0.22^{a}	82.71±0.60^{f}
10 % additive	Pp	43.51±0.11^{a}	8.21±0.32^{c}	17.75±0.39^{c}	52.75±0.97^{a}
	Ls	52.76±0.18^{d}	10.80±0.81^{e}	23.05±0.52^{e}	63.83±0.78^{d}
	SPF	48.45±0.19^{c}	9.31±0.74^{c,d}	21.89±0.64^{d}	66.96±0.88^{e}
	TP	42.14±0.17^{a}	4.32±0.37^{b}	12.32±0.60^{a}	52.65±0.55^{a}
30 % additive	Pp	45.64±0.11^{a}	19.09±0.21^{f}	28.16±0.69^{f}	55.87±0.96^{b}
	Ls	47.96±0.20^{c}	19.27±0.93^{f}	29.93±0.71^{g}	57.22±0.63^{c}
	SF	44.54±0.23^{b}	12.01±0.63^{e}	20.33±0.55^{d}	54.32±0.49^{a}
	TP	42.10±0.25)^{a}	7.56±0.41^{c}	14.24±0.44^{b}	53.66±0.86^{a}

Means in columns with different letters are significantly different ($p \leq 0.05$)
RCMP samples: Control – without additives, TP – tomato powder, SF – with spontaneously fermented TP, Pp – with *P. pentosaceus* fermented TP, Ls – with *L. sakei* fermented TP

Significant differences have been found in the intensity of taste, colour and acceptability of RCMP with different amounts of tomato by-product powder fermented with pure LAB or spontaneously fermented (Fig. 4.17). The acceptability scores of RCMP with 10 % of TP varied between 8.5 and 9.3. The lowest taste is in the RCMP samples with 30 % of fermented TP. The RCMP samples with 30 % of TP additives have the most intensive colour (5.5–6.5) (Fig. 4.17).

The RCMP with 10 % of TP fermented with *L. sakei* and *P. pentosaceus* have the highest scores of acceptability (Fig. 4.17). Acceptability scores of the control sample (without additives) are found to be lower by 34.2 and 5.6 %, respectively, than those with fermented TP. The RCMPs with 30 % of fermented TP have a lower acceptability (by 30.3 %) than those with 10 % of fermented TP, perhaps due to an undesirable acidic taste. However, the RCMPs with 30 % fermented of TP are rated as highly acceptable (an average of 7.7) (Fig. 4.17).

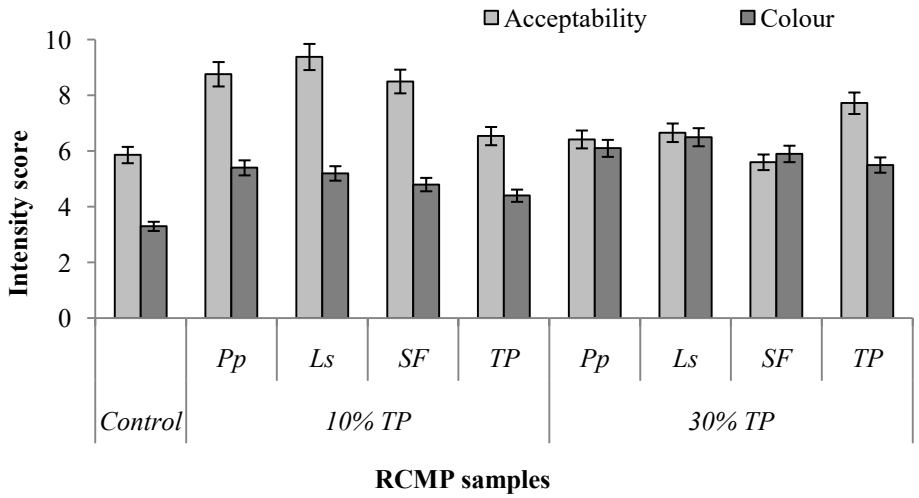


Fig. 4.17. Acceptability and colour intensity of RCMP prepared without and with tomato by-product powder (TP) additives. Samples: Control – without TP, TP – with tomato powder, *SPF* – spontaneously fermented, *Pp* – fermented with *P. pentosaceus*, *Ls* – fermented with *L. Sakei*

According to our results, the meat product colour parameters are significantly affected by the amount of TP added and the RCMP treatment. The addition of fermented TP does not negatively affect technological processing, so the RCMP with 10 % of fermented TP is a suitable meat product with the addition of biologically active substances, which also have a positive effect on the overall consumer acceptance.

4.5. Characterisation of tomato juice and different tomato-based juice blends fortified with isomerised lycopene extract

Functional foods generate one of the most promising and dynamically developing segments of the food industry, therefore, in this study, chemical and organoleptic assessment of tomato juice and different tomato-based juice blends fortified with isomerised lycopene extract was carried out in order to determine the most acceptable blend and the levels of lycopene isomers and other phytochemicals in such products.

Chemical analysis

The results of chemical analysis reveal that juice blends containing apple juice (TApl15%, TApl25%, TApl35%) have lower pH and higher titratable acidity compared with the control sample and tomato juice blends with carrots or sweet corn (TCar and TCor, respectively). Since sweet corn and carrots are generally low in acids, the tomato-sweet corn and tomato-carrot juice blends have higher pH values and lower titratable acidity. The pH value of juice with the highest percentage of sweet corn (TCor35%) is above 4.5, therefore such juice would require a more severe thermal treatment regime which is used for the processing of low acid foods.

Juice blends with apple, carrot, and sweet corn had higher TSS contents compared to the control juice (tomato juice). Compared to the control juice, the TSS content in TApl was higher by 22–59 %, in TCar – by 9–19% and in TCor – by 8–11%. Higher percentage of apple, sweet corn or carrot juice in the blends results in higher contents of TSS in the product. The content of ascorbic acid in the control juice was 8 mg/100 mL, whereas its content in juice blends in most cases was slightly lower. The lowest content of ascorbic acid (lower by 10–13%) was found in tomato-carrot juice blends.

The initial lycopene content in tomato juice was 7.94 mg/100 g, where *cis*-isomers constituted only 11.2 % of the total lycopene concentration. The initial content of β-carotene in tomato juice without extract additive was 0.96 mg/100 g. The addition of isomerised lycopene extract to tomato juice as well as juice blends significantly increase the levels of lycopene, especially the levels of *cis*-lycopene, which is more readily absorbed than all-*trans*-lycopene, in the final products (Table 4.12). The content of total lycopene in the juice blends varied from 16.21 mg/100 g (TApl35%) to 25.65 mg/100 g (TCor15%), whereas the content of cis-lycopene from 9.16 to 14.46 mg/100 g (in TApl35% and TCor15%, respectively). As expected, lower contents of lycopene were present in the blends with higher percentages of apple, carrot, and sweet corn juices. Tomato-carrot juices had the highest contents of β-carotene among the tested juice blends. Its content in TCar was 4.4–7.2 times higher compared to the control juice. The content of β-carotene in TCar gradually increased from 42.20 to 69.5 mg/100 mL as the percentage of carrot juice in the blend increased. It is interesting to note that an ingestion of β-carotene and lycopene, as mentioned before, improves the absorption of lycopene in humans (Johnson et al., 1997).

Table 4.12. The content of total lycopene and its isomers in tomato and juice blends with isomerised lycopene extract additive

Samples	Concentration mg/100g in juice			
	β–CAR	***trans*-LYC**	**Total *cis*-LYC**	**Total LYC**
Control of tomato juice	0.96± 0.030b,c	7.57±0.125b	0.37±0.031a	7.94±0.024a
TApl15%	0.93±0.106h	9.67±0.123e	12.67±0.040h	22.34±0.026g
TApl25%	0.74±0.135g	8.61±0.096d	11.27±0.047f	19.88±0.051e
TApl35%	0.63±0.127f	7.04±0.077a	9.16±0.057b	16.21±0.036b
TCar40%	4.67±0.014e	9.68±0.075e	12.37±0.020g	22.05±0.237f
TCar50%	4.22±0.015d	8.23±0.074c	10.96±0.015e	19.19±0.025d
TCar60%	6.95±0.007a	7.1±0.129a	9.54±0.040c	16.64±0.031c
TCor15%	1.17±0.047c	11.19±0.076g	14.46±0.030j	25.65±0.151i
TCor25%	0.98±0.015b,c	10.1±0.108f	12.95±0.050i	23.05±0.040h
TCor35%	0.75±0.026a,b	8.49±0.101c,d	10.81±0.035d	19.30±0.100d

Different letters in the same column indicate significant differences between the samples ($p \leq 0.05$).

Although the content of lycopene in the juice blends reduced with the addition of fruit and vegetable juices that initially contain no lycopene, it can be assumed that tomato-apple juice blends are enriched with health-beneficial compounds that are present in apple juice and normally are not found or found in negligible amounts in tomatoes, such as hydroxicinamic acids, dihidrochalcones, flavonols, catechins and oligomeric proanthocyanidins (Gerhauser, 2008). Oligomeric procyanidins have attracted increasing attention in the fields of nutrition and medicine due to their potential health benefits observed *in vitro* and *in vivo* (Aron, Kennedy, 2008). Similarly, tomato-sweet corn juice was enriched with biologically valuable constituents that are present in corns, such as ferulic acid, a potent antioxidant and anticarcinogenic agent (Balakrishnan et al., 2008).

Colour characteristics of juice

The estimated CIEL*a*b* parameters of tomato juice and juice blends are shown in Table 4.13. Tomato-apple juice blends had the lowest lightness (L*), redness (a*) and yellowness (b*) values compared with the control juice and juice blends with carrots and sweet corn. TCor25% and TCor35% juice blends were the lightest (L*=44.06 and 46.56, respectively) and had the highest yellowness values (b*=23.11 and 26.40, respectively).

Chroma (C*) is an expression of the purity or saturation of the colour. TCor blends presented the highest C*, followed by TCar, the control juice and TApl. The hue angle (h°) of juice blends with carrots, apples and sweet corn was lower compared with the tomato (control) juice. The h° value of the control juice was 44.83 indicating that the redness component had larger influence on the overall colour of the product (0° indicates that the juice is red and 90° indicates that the juice is completely yellow). The addition of apple, carrot juice and, in particular, sweet corn juice increased the influence of the yellowness component on the overall colour of investigated juice blends.

Table 4.13. CIEL*a*b* colour parameters of the control (tomato) juice and tomato-based juice blends

Parameters	a*	b*	a*/b*	L*	C*	h*	ΔE
Control	13.2±0.18d	13.2±0.20b	1±0.02db	42.4±0.03a	18.7±0.20c	44.8±0.53a	–
TApl15%	11.5±0.09c	12.1±0.07a	0.95±0.08ac	33.6±0.09a	16.7±0.11b	46.4±0.11b	8.9±0.69b
TApl25%	10.9±0.10b	12.1±0.21a	0.90±0.11ab	33.5±0.10a	16.2±0.09a,b	48.0±0.76c,d	9.3±0.10b
TApl35%	10.3±0.02a	11.5±0.05a	0.89±0.03a	33.5±0.03a	15.4±0.02a	48.2±0.18c,d	9.5±0.03b
TCar40%	15.3±0.09e	16.6±0.08c	0.92±0.08ce	36.9±0.02b	22.6±0.12d	47.3±0.02b,c	6.8±0.08a
TCar50%	16.3±0.01f	17.9±0.10d	0.91±0.05df	38.3±0.04c	24.1±0.07e	47.7±0.15c,d	7.0±0.04a
TCar60%	16.6±0.08f	18.8±0.17e	0.88±0.12ef	39.2±0.01d	25.1±0.15f	48.5±0.23d	7.3±0.14a
TCor15%	15.6±0.17e	19.9±0.27f	0.78±0.22ef	40.2±0.02e	25.3±0.12f	51.9±0.67e	7.5±0.19a
TCor25%	16.3±0.03f	23.1±0.15g	0.71±0.07fg	44.1±0.07g	28.3±0.14g	54.8±0.16f	10.6±0.16c
TCor35%	16.4±0.52f	26.4±0.82h	0.62±0.18fh	46.6±0.42h	31.0±0.96h	58.2±0.27g	14.5±0.15d

*Different letters in the same column indicate significant differences between the samples ($p \leq 0.05$).

The total colour difference (ΔE) values higher than 5 indicate large colour differences between samples that are easily perceived by an inexperienced observer. The total colour difference values indicate that the addition of apple, carrot, and sweet corn juice significantly changed the initial colour of the control juice. The lowest ΔE values had TCar (6.79–7.33), whereas the highest ΔE had TCor25% and TCor35% (10.55 and 14.25, respectively).

The principal components analysis use of classification the juice in taste

The principal components analysis (PCA) was used to classify the juice samples according to their similarity in taste. A PCA score plot for data obtained from the E-tongue is shown in Fig. 4.18. PC1 vs. PC2 together explain 85.927% of the variance. As it is seen, not all juice samples could be distinguished reliably with the help of PCA in two-dimensional principal components.

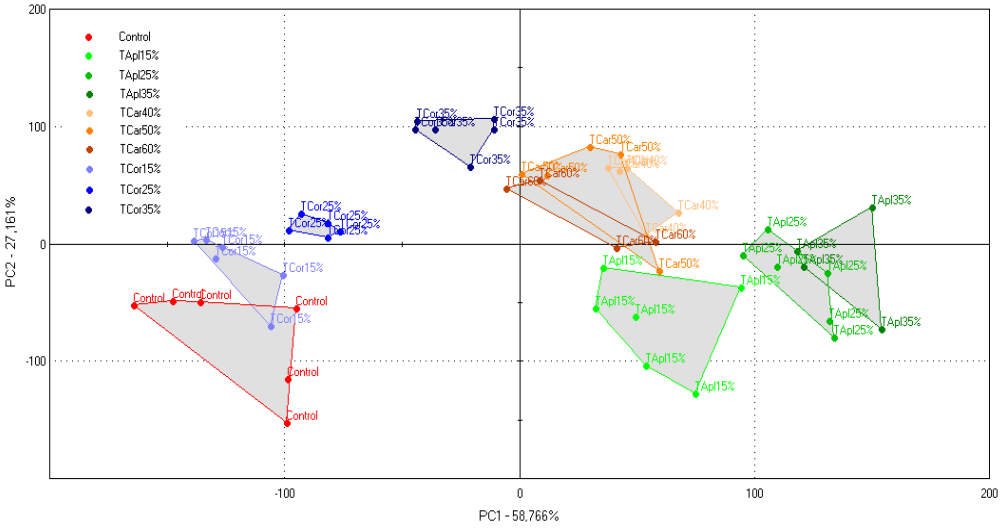


Fig. 4.18. Discrimination of PCA plots for tomato (control) juice and different tomato-based juice blends

The PCA scores plot on the PC1 and PC2 shows an overlap of tomato-carrot juice samples (TCar40%, TCar50%, TCar60%) and two tomato-apple juice samples (TApl25%, TApl35%). This indicates that the overlapping samples are quite similar in taste and that the E-tongue could not differentiate between those samples. However, as it is seen from the PCA graph, there is a distinct discrimination among the tomato-sweet corn (TCor15%, TCor25%, TCor35%), tomato-carrot (TCar40%, TCar50%, TCar60%) and tomato-apple clusters (TApl15%, TApl25%, TApl35%) showing that different tomato-based juice blends taste very differently. The juice samples that are closer to one another in the PCA score plot share more similar taste characteristics, according to which the tomato-sweet corn juice blend with the lowest amount of sweet corn juice (TCor15%) is the most similar to the control sample (tomato juice), whereas the most distinct from the control sample (furthest clusters from the control) are the tomato-apple juice samples.

The influence on sensory characteristics of the juice

Consumer acceptance of functional foods is far from being unconditional, with one of the main conditions for acceptance pertaining to taste. It is important to recognize that functional benefits may provide added value to consumers but cannot outweigh the sensory properties of foods (Siro et al., 2008). Therefore, sensory evaluation was performed in order to select the best tasting functional tomato-based juice blend. The results of the acceptability test are shown in Table 4.14.

Table 4.14. The acceptability scores of tomato-based juice blends

Samples	Taste	Odor	Appearance	Consistency
TApl15%	3.0±0.38a,b	3.3±0.32 a,b,c	4.5±0.19c,d	3.0±0.38 a,b
TApl25%	3.8±0.42b	3.8±0.40b,c	4.5±0.19c,d	3.5±0.43 b,c
TApl35%	4.8±0.17c	4.0±0.27b,c	4.3±0.17b,c,d	4.5±0.19d
TCar40%	3.8±0.32b,c	4.0±0.32b,c	4.0±0.27b,c	4.0±0.27c,d
TCar50%	3.5±0.33b	4.1±0.22c	4.5±0.18c,d	4.1±0.09c,d
TCar60%	3.3±0.32a,b	3.3±0.32a,b,c	4.8±0.16d	3.5±0.33b,c
TCor15%	3.3±0.42a,b	3.8±0.42b,c	4.0±0.25b,c	3.3±0.32a,b,c
TCor25%	3.0±0.47a,b	3.0±0.54a,b	3.8±0.32b	3.0±0.47a,b
TCor35%	2.3±0.42a	2.3±0.32a	2.8±0.29a	2.5±0.19a

*Different letters in the same column indicate significant differences between the samples ($p \leq 0.05$).

According to the results, the tomato-apple juice blend that contained 35% of apple juice had the most acceptable taste, followed by the tomato-carrot juice blend that contained 40% of carrot juice. The tomato-sweet corn juice with the highest percentage of corn juice (35%) was one of the least preferred. Some panellists commented on TCor35% blend as being pungent. The TCar50% blend ranked the highest for odour acceptability, followed by TCar40%, TApl35%, TApl25% and TCor15% blends which also received quite high odour acceptance scores (between 4 and 3.75 points). The appearance of TCar60% blend was most preferred to that of the other blends. The appearance of TApl15%, TApl25%, TApl35%, TCar50% was fairly liked, whereas the appearance of TCor35% and TCor25% was the least acceptable to the panellists, who reported TCor35% and TCor25% blends as being too light-coloured. The results of the Tukey's test show that the consistency of the TApl35% blend was the most preferred by the panellists. The consistency of TApl25%, TCar40%, TCar50% and TCar40% blends was fairly acceptable, whereas the consistency of TCor35% blend was the least preferred. Most of the panellists named TCor30% and TCor25% blends as being too thick.

4.6. A study of anti-inflammatory and antiproliferative properties of extract with isomerised lycopene

As mentioned in the literature review, biochemical reactions that take place in the cells and organelles of human bodies 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 leads to a state known as oxidative stress, inducing damage to the DNA, proteins and lipids. The balance between the production and neutralization of ROS by antioxidants is very delicate, and if this balance shifts towards the overproduction of ROS, the cells start to suffer the consequences of oxidative stress (Wiernsperger, 2003).

NADPH oxidases are the only enzymes whose primary function is to generate ROS, who are toxic to pathogens, but also damage the surrounding normal tissues and cells, causing various abnormalities, such as infection, arteriosclerosis, neurodegenerative diseases and inflammation. Biologically active compounds with plant origins have anti-inflammatory properties; the mechanisms of their action are not yet fully understood.

The aim of this study is to evaluate the anti-inflammatory effects of lycopene extract by assessing the production of hydrogen peroxide in a mouse's macrophage J774 cell culture. The production of hydrogen peroxide by macrophages was stimulated with arachidonic acid (ARA) and phorbol-12-myristate-13-acetate (PMA).

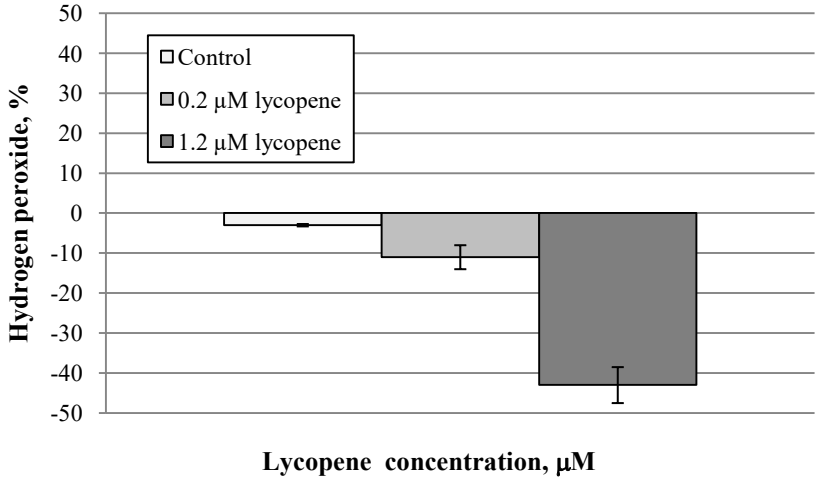


Fig. 4.19. Indirect effect of lycopene on the production of hydrogen peroxide in a J774 macrophage cell culture. Expressed as mean ± SE (n=3)

The effect of lycopene extract on the mouse J774 macrophage cell culture was evaluated in an experimental model. A selected concentration of lycopene corresponds to plasma-determined lycopene concentrations (0.2–1.5 µM in accordance with various authors) (Sarkar, Gupta, Salm, 2012; Shierle et al., 1997).

PMA was chosen for the activation of ROS generation in macrophages; PMA induces protein kinase C, which activates NADPH oxidase and triggers oxidative burst. Great amounts of ROS generated by NADPH oxidases participate in various inflammatory processes. The results demonstrate that, in this model, the lycopene isomer extract at the concentrations of 0.2 μM and 1.2 μM decreases the production of hydrogen peroxide by 11% and 43%, respectively (Fig. 4.19).

In direct experiments, the extract of lycopene isomers at concentrations of 0.2 μM and 1.2 μM inhibit the generation of hydrogen peroxide by 15–18%, while concentrations exceeding the levels in plasma (7 μM) promote the concentration of hydrogen peroxide by 20%. 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 (Fig. 4.20).

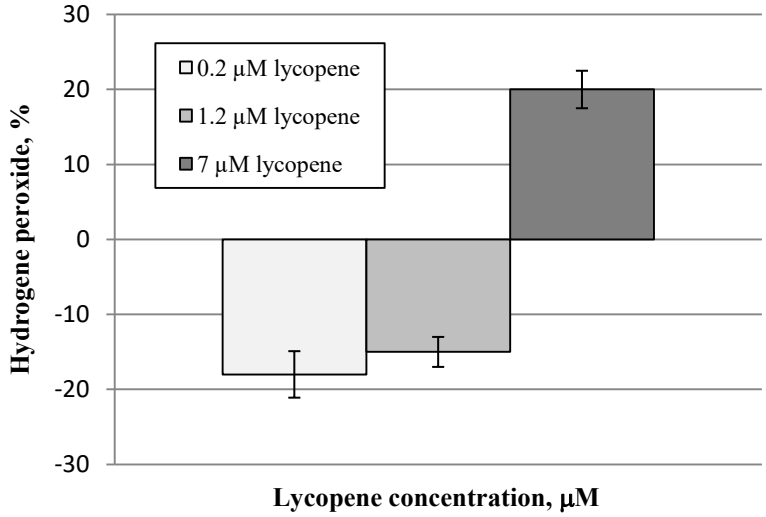


Fig. 4.20. Direct effect of lycopene extract on the generation of hydrogen peroxide in a J774 macrophage cell culture

In order to investigate the effect of lycopene on the viability of cancer cells, the rat glioblastoma C6 cells were chosen. Glioblastoma is one of the most aggressive and the most common glioma (malignant brain tumours) forms (Lim et al., 2011). Exact causes of glioblastoma initiation are not known but tumour development may be influenced by ionizing radiation, electromagnetic fields, infection and other undetermined causes. Research on the impact of lycopene isomer extract concentrations (0.05–2.4 μM) on glioblastoma cells determined that these concentrations inhibit cell viability by 20–42% (Fig. 4.21). The lowest effect was determined by 0.05 μM of lycopene isomers and the greatest effect (-42%) was determined by 2.4 μM of lycopene isomer extract.

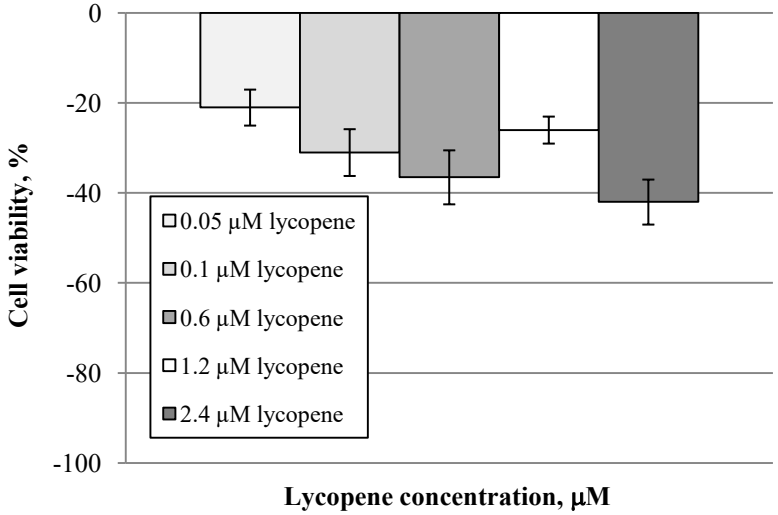


Fig. 4.21. The effect of lycopene on the viability of rat glioblastoma cells

In order to evaluate the effect of lycopene on the generation of intracellular ROS in glioblastoma cells, the cells were treated with different concentrations of lycopene (0.05µM–2.4 µM). We determined that lycopene promotes ROS burst in glioblastoma cells by 12%, 22%, 34% and 42% (Fig. 4.22).

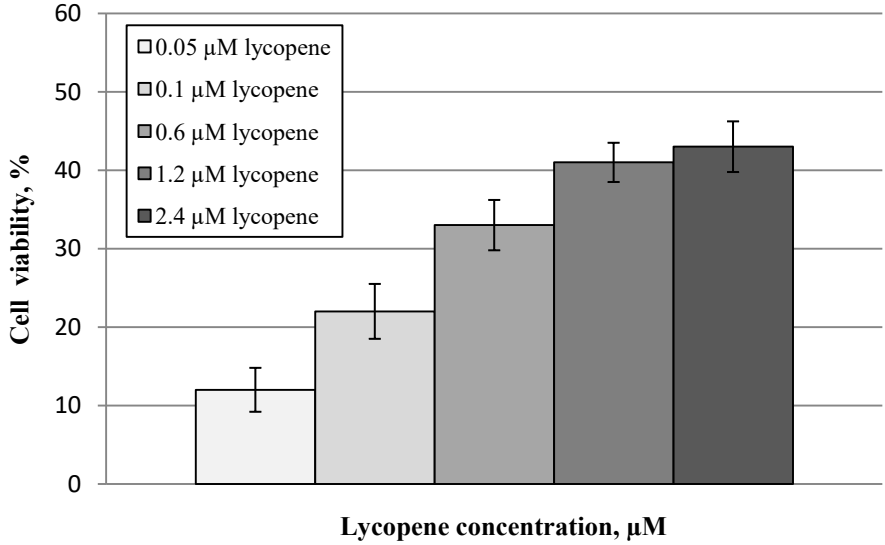


Fig. 4.22. The effect of lycopene on intracellular ROS generation in a rat glioblastoma cell culture

The effect increases depending on the concentration. The greatest effect (42%) was determined using the maximal investigated concentration of 1.2–2.4 μM. While it is known that the production of reactive oxygen species involves healthy cell signalling pathways and the alteration in redox status may result in the development of cancer, reactive oxygen species may also be used for killing tumour cells (Wang, 2008). Our results demonstrate that lycopene triggers the generation of ROS in glioblastoma cells and inhibits their viability. Whereas in a macrophage culture, the generation of ROS triggered by NADPH oxidases is promoted and may be associated with the anti-inflammatory effect 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, other researchers raise a hypothesis that lycopene could exert inhibition of carcinogenesis by several mechanisms: scavenging of reactive oxygen species, enhancement of detoxification systems and suppression of cell cycle progression as a modulation of signal transduction pathways. Therefore, *cis*-lycopene isomers are regarded as offering 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_2O_2 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, Hosokawa, Miyashita, 2013). There are several interesting studies concerning the effect of lycopene on protection against oxidative damage. Zini et al. (2010) preincubated human sperm samples with 0.2 or 5 μM lycopene before treating with H_2O_2 (50 μM) and established protection against DNA SBs (measured with the comet assay) at higher concentration. Under Seo et al. (2009), "Ku70 protein" involved in the repair of double-strand DNA break and its concentration decreased in rat pancreatic cells by oxidative stress induced by glucose/glucose oxidase, and the decrease was prevented by co-incubation with lycopene. The findings from this and other studies might suggest that cell culture studies might contribute useful information towards understanding the beneficial effects of carotenoids, especially their biologically more active forms, such as *cis*-lycopene isomers on human health.

In conclusion, the *in vitro* experiments demonstrated that the investigated lycopene isomer extract (depending on the concentration of lycopene) reduces the production of hydrogen peroxide in a J774 macrophage 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 hydrogen peroxide by 43%.

Our results reveal the cytotoxic effect of lycopene-rich *cis*-isomer (60%) extract on rat glioblastoma C6 cells. The viability of the glioblastoma C6 cells significantly decreased (-42%) at a total lycopene concentration of 2.4 μM after 24 h of incubation. 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 anti-inflammatory and antiproliferative 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.

4.7. Agro-refinery of tomato (*Lycopersicon esculentum*) fruits

After the production of juice of red-ripe tomato (*Lycopersicon esculentum*) fruits, residues (peels, seeds) in most cases are considered as by-products. The industrial processing of tomatoes into tomato products generates large amounts of by-products, such as peel, pulp, seeds and vascular tissues. These by-products create a major disposal problem for the industry in terms of costs and potential negative environmental impacts. However, they also represent a promising low-cost source of carotenoids (primarily lycopene), which may be used in functional foods due to their favourable nutritional properties. Studies have reported that tomato peel contains high levels of lycopene, about five times more than that of pulp and seeds (Sharma and Le Maguer, 1996). Therefore, the agro-refinery of tomato by-products for the recovery of valuable functional ingredients remains an important task. A diagram of tomato (*Lycopersicon esculentum*) fruits agrorefinery used in our study is presented in Figure 4.23.

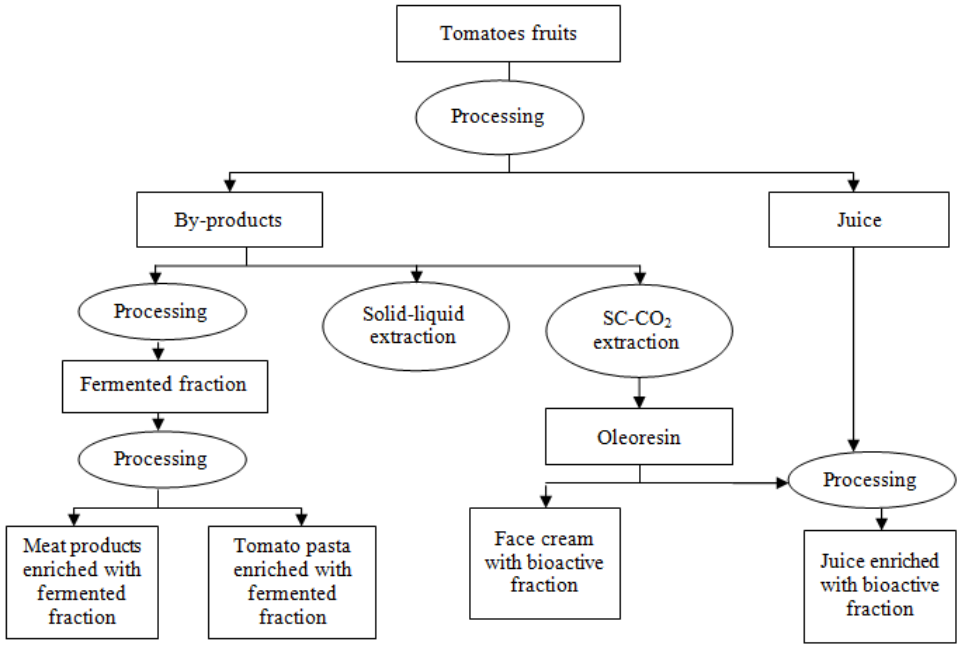


Fig. 4.23. An agro-refinery diagram of *Lycopersicon esculentum* fruits

5. CONCLUSIONS

1. High performance liquid chromatography method for the qualitative and quantitative analysis of lycopene and lycopene *trans*- and *cis*-isomers (*5-cis; 7-cis; 9-cis; 13-cis; 15-cis*) in tomatoes, tomato products and different isolated fractions from tomatoes was developed and validated with respect to precision, linearity and selectivity, and confirms the value of the methodology to analyse lycopene, lycopene isomers and β-carotene. The best chromatographic conditions were obtained with the RP-C30 (5μm, 250x4.6 mm) column, MeOH/TBME mobile phase gradient (flow rate 0.60 mL/min), at 473 nm detection.

2. The optimum parameters for the extraction of oleoresin from tomato by-products include 53.7 MPa pressure, 73.9 °C temperature and 155 min time, whereas the optimal extraction parameters of *cis*-lycopene isomers are 55 MPa pressure, 52 °C temperature and extraction time 180 min. The total oleoresin yield of tomato by-products was 251.2 g/kg DW. The dominant geometrical lycopene isomers in the extract are *9-cis* and *5-cis*. The total content of *cis*-lycopene isomers in the SC-CO_2 extract was 62% of the total lycopene content, showing that the SC-CO_2 extraction parameters could be modified to increase the concentration of c*is*-lycopene isomers in the extract.

3. The storage conditions (temperature from +1 to +37) and different light irradiation (200–2500 Lux) affects the stability of lycopene in tomato-based food model system. The *trans-cis* isomerization and *trans*-lycopene degradation occurred during the accelerated shelf-life storage. The isomerization rate of lycopene significantly increases at 37 °C causing the formation of 6 identified lycopene *cis*-isomers (*5-cis*, *7-cis, 9-cis, 13-cis* and *15-cis*), whereas *trans*-lycopene degradation in a tomato-based food model system increases with UV irradiation. The content of *5-cis*-isomer increases the most significantly (from 0 to 7.29 mg/mL) during shelf-life storage compared to other lycopene isomers (*7-cis, 9-cis, 13-cis* and *15-cis*). The highest stability of lycopene was observed at +1 °C in absence of light.

4. The fermentation of tomato by-products with selected lactic acid bacteria (*Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7, *Pediococcus pentosaceus* KTU05-8) affects the recovery of total carotenoids (especially lycopene) from the plant matrix. The recovery of lycopene from tomato by-products is most significantly increased (up to 37 %) by the lactic acid fermentation with recognized as safe *Lactobacillus sakei* KTU05-6.

5. The added extract (5%) with isomerised lycopene in the juice blends and fermented tomato fractions in minced meat products increases the content of lycopene and, especially, the percentage of its *cis*-isomers, thus improving functional properties of the products:

a) the tomato-apple juice blend (35% apple juice) and tomato-carrot juice blend (40% carrot juice) with increased *cis*-lycopene content had the most acceptable taste.

b) the addition of 10% of fermented tomato products to minced pork meat improves the colour and enhances the nutritional value of the final product without compromising the consumers' acceptance**.** The content of lycopene in the prepared minced meat products was 2.2 and 2.6 mg/100 g (product with *Lactobacillus sakei* KTU05-6 and *Pediococcus pentosaceus* KTU05-8 fermented tomato fraction, respectively).

6. The extract of isomerised lycopene (60% *cis*-isomers from the total content of lycopene) decrease generation of reactive oxygene species and viability of glioblastoma cells. The extract of isomerised lycopene decrease production of hydrogen peroxide by 43% in a J774 macrophages culture and decrease the viability of rat glioblastoma C6 cells by 42%. The concentrated extracts of isomerised lycopene could be applied as pharmacological agents possessing anti-inflammatory and anti-proliferative activities.

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Recommendation

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SL344. 2017-05-08, 14,75 leidyb. apsk. l. Tiražas 12 egz. Užsakymas 158.

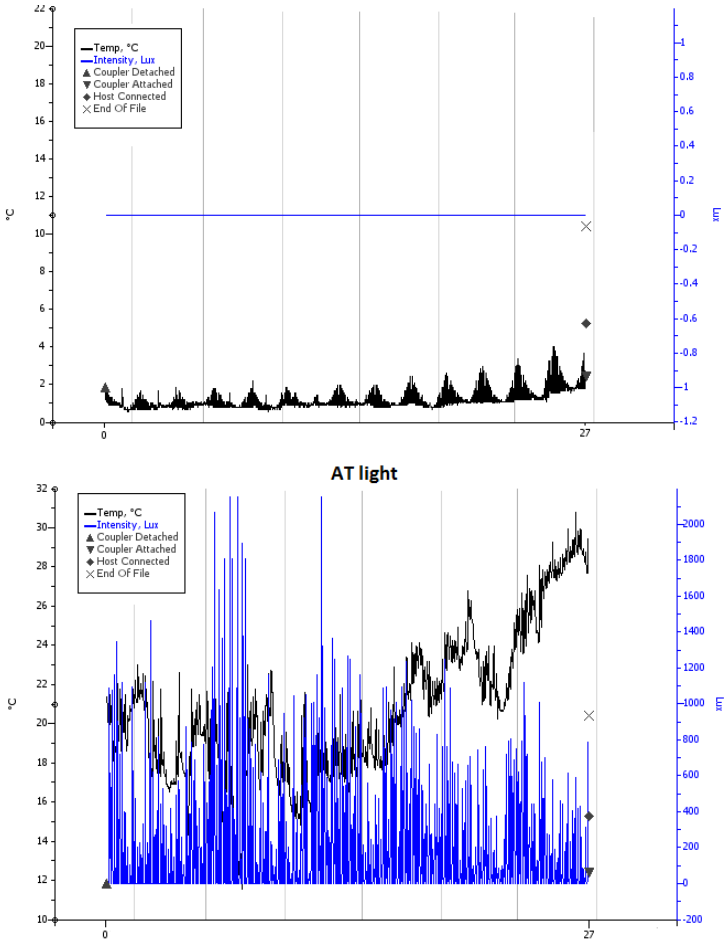
Išleido Kauno technologijos universitetas, K. Donelaičio g. 73, 44249 Kaunas

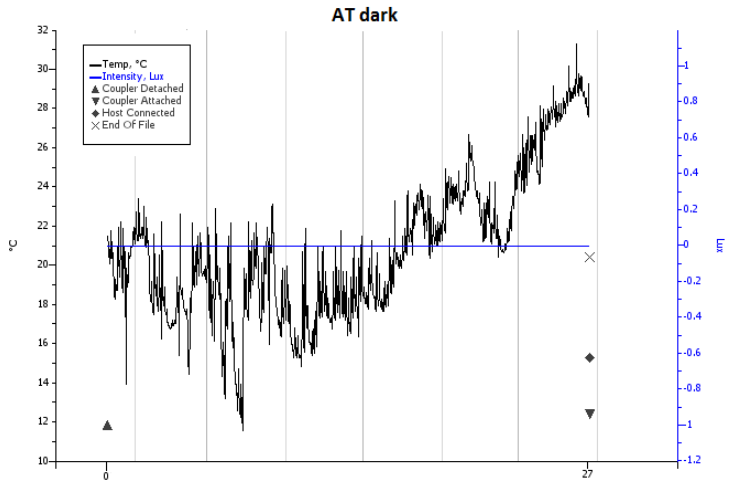
Spausdino leidyklos „Technologija" spaustuvė, Studentų g. 54, 51424 Kaunas

8. SUPPLEMENTS

Supplement 1: Storage conditions of a samples during a 200-day period

FT dark – fridge temperature at 1 ± 1°C in absence of light; AT light – ambient temperature at 20 ± 1°C in natural light with different day and night illumination (average illumination 200 ± 10 Lux); AT dark – ambient temperature at 20 ± 1°C in absence of light (0 Lux); TT dark – thermostatically controlled temperature at 37 ± 1°C in absence of light (0 Lux); TT UV – thermostatically controlled temperature at 37 ± 1°C in UV irradiation (2500 ± 100 Lux)

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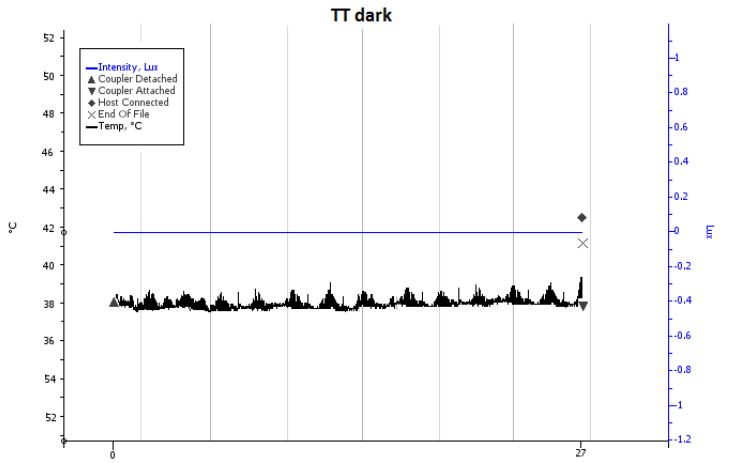
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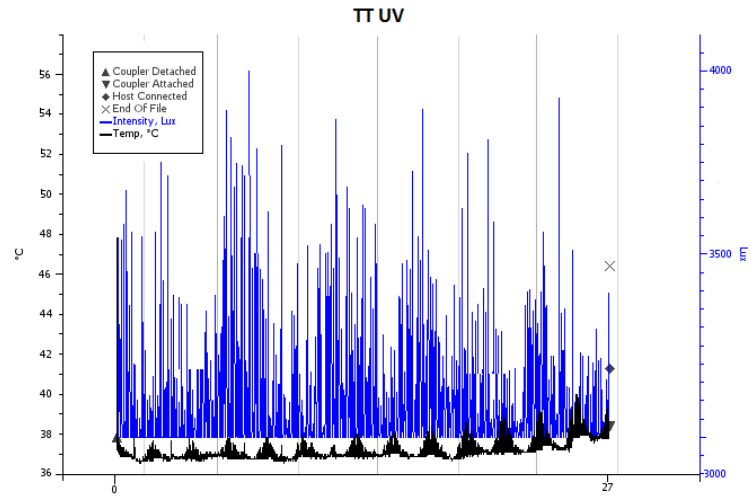
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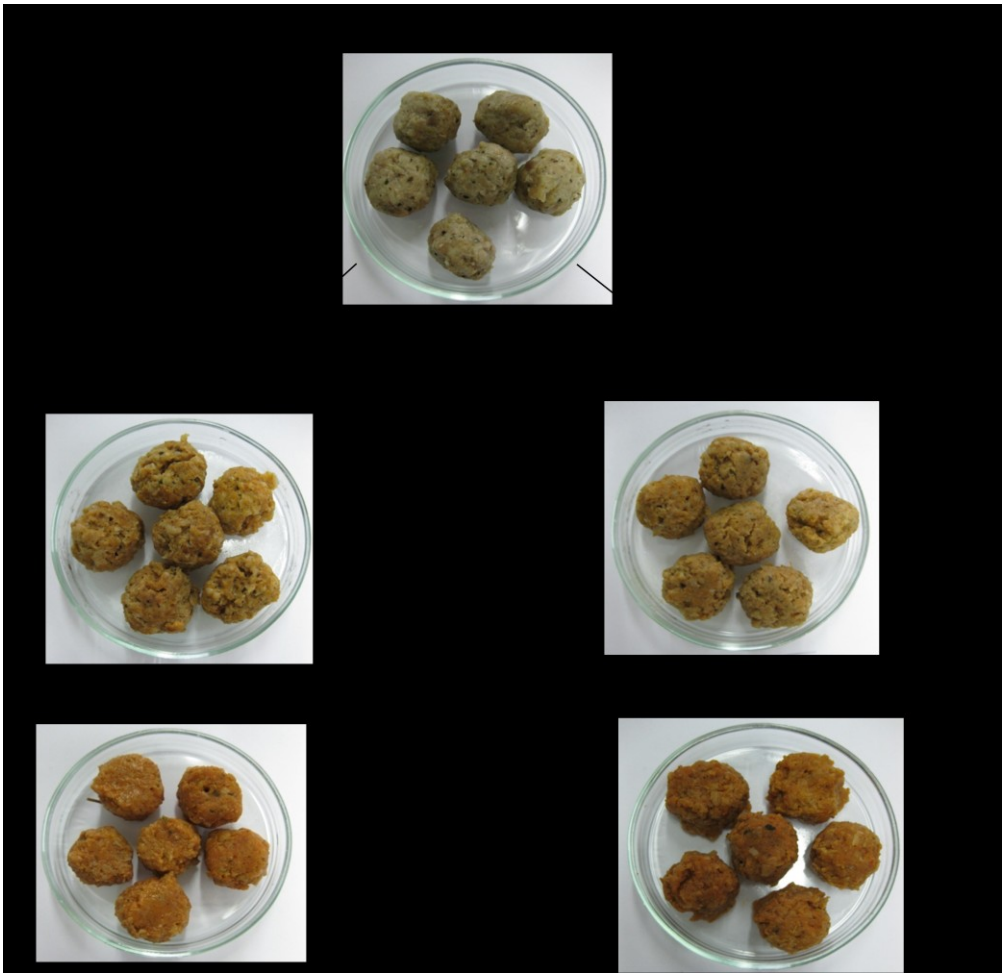
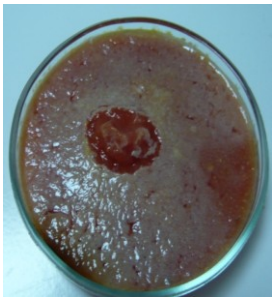
Supplement 2: Prototypical product with lycopene *cis*-isomers – noisettes with fermented tomato powder with *Pediococus pentosaceus MI807* and *Lactobaccilus sakei*



Supplement 3: The tomato fermentation with different kinds of LAB compared with not fermented and spontaneously fermented samples



Pediococcus pentosaceus KTU05-8

Lactobacilus sakei KTU05-6

Spontaneous sourdough

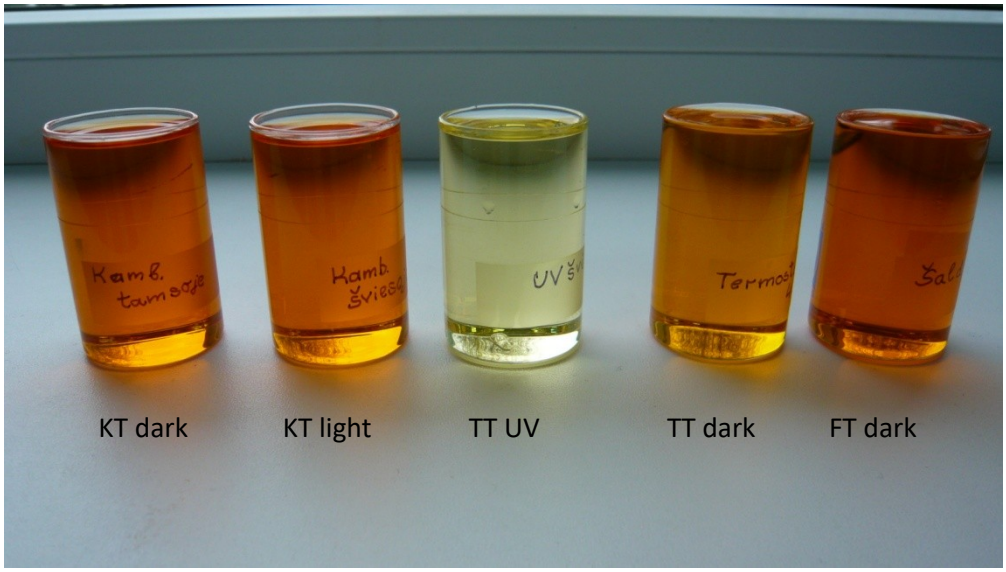
Supplement 4: The prototypical product – tomato juice and different tomato-based juice blends fortified with isomerised lycopene extract

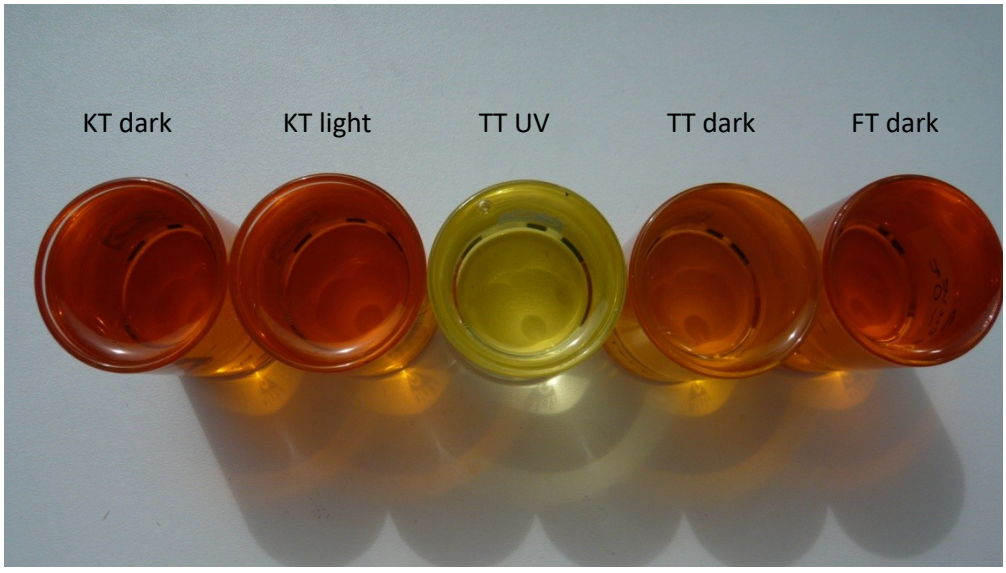
Tomato-apple juice blends 85:15, 75:25 and 65:35, v/v (TApl15%, TApl25%, TApl35%, respectively):

Tomato-carrot juice blends 60:40, 50:50 and 40:60, v/v (TCar40%, TCar50%, TCar60%, respectively):

Tomato-sweet corn juice blends 85:15, 75:25 and 65:35, v/v (TCor15%, TCor25%, TCor35%, respectively):

Supplement 5: Lycopene in an oil-based food model system





Supplement 6:

LIETUVOS AGRARINIŲ IR MIŠKŲ MOKSLŲ CENTRO SODININKYSTĖS IR DARŽININKYSTĖS INSTITUTE BENDRADARBIAUJANT SU UAB „BIOKOSMETIKOS MANUFAKTŪRA" SUKURTA VEIDO KREMO RECEPTŪRA SU POMIDORŲ IŠSPAUDŲ ALIEJUMI, KURIAME DOMINUOJA LIKOPENO *CIS*-IZOMERAI, TECHNOLOGIJA IR ĮDIEGTA Į GAMYBĄ

AKTAS Nr. 23

2016 m. balandžio 18 d.

Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės instituto Biochemijos ir technologijos laboratorijoje UAB „BIOKOSMETIKOS MANUFAKTŪRA" užsakymu buvo atlikti moksliniai užsakomieji tyrimai, siekiant sukurti receptūras veido kremui su pomidorų išspaudų aliejumi, kuriame dominuoja *cis*-izomerai, atlikti stabilumo, biocheminiai, fizikocheminiai ir mikrobiologiniai tyrimai. Mokslo darbuotojai Dalia Urbonavičienė ir Pranas Viškelis sukūrė ir įdiegė į gamybą **veido kremo su pomidorų išspaudų aliejumi, kuriame dominuoja *cis*-likopeno izomerai, technologiją.** Technologija įdiegta 2015 – 2016 metais UAB „BIOKOSMETIKOS MANUFAKTŪROS" užsakymu.

Veido kremo su pomidorų išspaudų aliejumi, kuriame dominuoja *cis*-likopeno izomerai technologija įdiegta į gamybą:
UAB „Biokosmetikos manufaktūra" direktorė Birutė Nastaravičiūtė

Supplement 7:

LIETUVOS AGRARINIŲ IR MIŠKŲ MOKSLŲ CENTRO SODININKYSTĖS IR DARŽININKYSTĖS INSTITUTE BENDRADARBIAUJANT SU UAB „MĖLYNĖ“ SUKURTA RECEPTŪRA POMIDORŲ-OBUOLIŲ SULČIŲ, PRATURTINTŲ LIKOPENO EKSTRAKTU SU PADIDINTU *CIS*-IZOMERŲ KIEKIU, ŠI TECHNOLOGIJA ĮDIEGTA Į GAMYBĄ

AKTAS Nr. 20

2016 m. vasario 9 d.

Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės instituto Biochemijos ir technologijos laboratorijoje buvo atlikti moksliniai užsakomieji tyrimai, siekiant sukurti receptūrą pomidorų-obuolių sulčių, praturtintų likopeno ekstraktu su padidintu *cis*-izomerų kiekiu, ir atlikti stabilumo, biocheminių ir fiziknių savybių nustatymo, bei mikrobiologini tyrimai. Mokslo darbuotojai Dalia Urbonavičienė ir Pranas Viškelis sukūrė ir įdiegė į gamybą **pomidorų-obuolių sulčių, praturtintų likopeno ekstraktu su padidintu *cis*-izomerų kiekiu technologiją.** Technologija įdiegta 2015 – 2016 metais UAB „MĖLYNĖ“ užsakymu.

Pomidorų-obuolių sulčių, praturtintų likopeno ekstraktu su padidintu *cis*-izomerų kiekiu technologija įdiegta į gamybą:

UAB „Mėlynė“ direktorė Nijolė Karpavičienė .....