Enhanced Lycopene Extraction from Tomato Processing Residue Using Oil-in-Water Emulsions

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Abstract

Tomato is one of the most important crops worldwide. According to the Food and Agriculture Organization (FAO), the total global production of tomatoes was approximately 180 million ton in 2018. Forty million ton of tomatoes are processed as juice or paste. Generally, tomato processing residue is produced during processing these products, which consists mainly of peels and seeds. In these circumstances, the total estimated amount of tomato processing residue in the world is 5.4×10^6 ton. Tomato is very rich in the natural antioxidant compounds lycopene (5 mg/g). Tomato peels and seeds hold the greatest quantity of lycopene (588 µg/g). However, tomato processing residue can potentially be considered an interesting source of lycopene. The purpose of this research was to extract lycopene from tomato processing residue using an organic solvent, triglyceride, fatty acid, and oil-in-water (O/W) emulsion with or without an emulsifier. The effects of different temperature and time on lycopene recovery and the chemical stability of lycopene in the extract was also evaluated.

Two types of O/W emulsions were used for emulsion-assisted extraction: (a) O/W emulsion with or (b) without 0.1% (w/w) of saponin as a natural emulsifier. This environment-friendly extraction method was compared with the conventional method using an organic solvent, triglyceride, and fatty acid.

Lauric acid-based O/W emulsion without emulsifier resulted in the highest lycopene recovery 73% (Initial lycopene content: 588 µg/g). Moreover, lauric acid-based O/W emulsion extraction enhanced lycopene recovery two times higher compared to organic solvent triglycerides, respectively. Oil-in-water (O/W) emulsion consists of a large amount of a protic solvent water, which can both donate and absorb hydrogen bonds. This activity may assist to swell the tomato residue's cell wall and promote lycopene recovery. This is because three solvent characteristics

have a significant impact on the swelling process: basicity, hydrogen bonding ability, and molar volume. The high polarity and hydrogen bonding ability of solvent molecules also help the substance to swell. Therefore, high polarity (12.04 MPa $^{0.5}$) and hydrogen bonding ability (31.17 MPa $^{0.5}$) of lauric acid-based O/W emulsion helps to penetrate the plant matrix, which enhances lycopene recovery compared to hexane and soybean oil. On the other hand, the use of natural emulsifier saponin for preparing the O/W emulsion reduced the lycopene recovery compared to without an emulsifier. The decrease in lycopene recovery might be linked to the creation of a physical barrier at the droplets' interface, preventing lycopene from penetrating into the triglycerides, or fatty acid droplets' core. On the other hand, 77% lycopene recovery with lauric acid O/W emulsion was investigated at 50 ˚C at 2 h extraction. Moreover, lycopene in the lauric acid extract was stable until 6 days of storage at 5 ˚C.

In this study, O/W emulsion without an emulsifier was efficient to enhance lycopene recovery. Moreover, an emulsifier may inhibit lycopene from partitioning into triglycerides and fatty acids. Lauric acid shows the highest recovery as a non-toxic and green solvent compared to other solvents. Furthermore, a lauric acid-based O/W emulsion has the considerable benefit of being able to be used directly as a food additive once developed with the recovered lycopene.

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Chapter 1

General Introduction

1.1 Tomato

Tomato is the edible fruit which is commonly known as a tomato plant. It belongs to the nightshade family. Various types of tomatoes are widely grown in temperate climates across the world. It grows as an annual in temperate areas but is a perennial in its natural environment. Tomatoes may also grow in greenhouses, enabling year-round production in milder climates. The plants normally reach a height of 1-3 meters. After the Spanish conquest of the Americas, it expanded around the world from its Mexican origins. Tomato is used in various cuisines, sauces, salads, and beverages in a variety of forms, including raw. It is considered culinary vegetables, being ingredients of savory meals, while tomatoes are botanically berry-type fruits.

Moreover, for both commercial and domestic purposes, tomatoes are one of the most widely grown plants in the world and Food and Agriculture Organization mentioned that their global production reached 180 million tons in 2018. Its main production locations are mostly in the Northern Hemisphere. China is one of the largest producers, accounting for almost a 3rd of world output, followed by India, Turkey and the United States (Gauld, 1992).

Tomatoes are consumed fresh, cooked, sun-dried, or pickled in the domestic. At the food business level, 40 million ton of tomatoes are handled every year, with consumption predicted to approach 50 million tons by 2025. Tomato juice, Canned tomatoes, paste, tomato soup, puree, ketchup, , dehydrated pulp and dried tomatoes are all commercial products. However, industrial tomato processing produces a significant amount of processing residue, which accounts for around 5% of the processed tomatoes by weight and generates almost 4 million ton of organic materials. Tomato peels and seeds, as well as a tiny proportion of pulp remnants, comprise "tomato processing residue." Tomato processing residue is considered a concern for the tomato business since it has little economic value and is, in most circumstances, underused, dumped as trash, or

used to a limited level as animal feed. However, if properly treated, tomato processing residue may be a rich source of nutrients, bioactive compounds, and important phytochemicals including carotenoids, phenolic compounds, and lycopene. (Domínguez et al., 2020).

1.2 Processing of tomato

The tomato (Solanum Lycopersicon), which currently ranks in regard to production as well as in terms of operation, is the second most eaten fruit or vegetable. In households, the food industry, and restaurants, fresh or processed tomatoes or fresh are frequently utilized. Around 40 million tons of tomatoes are processed annually in the worldwide tomato processing sector. Sauce, ketchup, juice Paste, and diced canned and whole peeled tomatoes are the most popular processed tomato products (Figure 1.1). The mass of the tomatoes used in manufacturing are turned into a large volume paste; the remainder is used to make diced or whole peeled tomato products. (Knoblich et al., 2005).

1.3 Tomato processing residue

The tomato industry has a severe problem with tomato processing residue, which includes both solid and liquid pollutants. Clean-up water, cooling water, chemical peel water and peeling solutions, wash waters, , and are examples of solid wastes, as opposed to liquid wastes, which involves clean-up waters, peel water and chemical peeling solutions, cooling water, and wash waters. Tomato seeds comprise around ten percent of the fruit, while they account for 50 to 55% of the residue. The quantity of tomato processing residue manufactured yearly varies depending on the qualities of the tomatoes and the processing circumstances, and varies from 5% to 13% of the total mass of the tomatoes, with thousands to 2 million ton of waste product discarded away (Knoblich et al., 2005). Culled tomato, tomato seeds cake and leaves are examples of other tomato processing residue. Culled tomatoes are vegetables that don't live up to match customer

expectations owing to imperfections in appearance (color, size, shape, etc.). Roughly 2% of the harvest is eliminated as tomato. Seed cake of tomato is a good source of riboflavin, calcium, proteins, phosphorus and magnesium. It is formed after the lipid from tomato fruits is extracted. Tomato processing waste is now valued by being used as a component in animal feed stuffs or as pesticide as well as being disposed of in hills (Tsatsaronis & Boskou, 1975). The oil industry uses only a small portion of the seeds. The tomato processing sector has substantial economic and environmental challenges regarding the disposal of this residue. Because tomato processing residue contains magnificent substances such as dietary fibers, carbohydrates, phenolic compounds, fatty acids, and proteins that stimulate social wellbeing The fruit industry is working to solve this issue by creating a system that effectively manages output in aspects of reuse and utilization (Gould et al, 1992). Lycopene is the most studied of the minor tomato processing residue components due to its possible health advantages.

1.4 Sustainable applications of tomato processing residue

Different approaches for the long-term use of tomato processing residue have been presented and detailed in the following paragraphs, based on the economic concept provided by the European Commission. According to this perspective, natural elements from the water and soil should be utilized as inputs to the manufacturing of food and nutrition in advanced nations as well as industrial and energy production. In addition, for the creation of sustainable products, the manufacturing should be dependent on the usage of biodegradable resources. Thus, such long-term use of vegetables/fruits waste for the extraction of valuable compound with potential uses in the agriculture, nutrition, biotechnology, and pharmaceutical sectors might aid in addressing 21st-century societal concerns (Pinela et al., 2017).

Foods made with wheat flour have the most potential for incorporating tomato processing residue and its components. Protein, dietary fiber, and lycopene levels have all increased in all relevant studies. Furthermore, adding up to 10% tomato processing residue powder to bread did not have any negative effects on its acceptance. However, because of the lower gluten levels, the majority of quasi flours, a reduction in sample capacity is predicted. Other findings revealed that incorporating 5% wt tomato processing residue powder into cookies had no significant influence on overall cookie attractiveness scores, however, tomato processing residue concentrations up to 25% wt had a negative effect. The presence of furostanol saponin in tomato processing residue induced an increase in bitterness in crackers containing tomato processing residue powder. The results demonstrate that incorporating tomato processing waste into meals based on flour is possible, although the impacts are reliant on the amount of tomato processing residue used and the overall composition of the items (Lu et al., 2019).

Additionally, tomato processing residue has been found in meat items including ham, sausages, and hamburgers. Because tomato processing residue may be a natural colorant, the resultant products were not only high in antioxidants and nutritional composition but also had a further attractive brightness. Additionally, tomato processing residue filaments can alter every morphological aspects; tomato peel pectin can boost midst, while lignin and cellulose impact flexibility and stability, causing in greater stringy (Lu et al., 2019). Finally, using tomato processing waste as a component of tomato paste is highly suggested. Up to 2% weight of powdered and dried tomato processing residue may be added to paste of tomato. The flavor and expansion of the produced paste of tomato are not negotiated or destructively impacted, according to relevant research, and are equivalent to commercial tomato paste. Higher β-carotene and lycopene concentration in final products is also possible (Lu et al., 2019). Because of the extra

group functioning (e.g., crushing, dehydrating) necessary for its preparedness, tomato processing residue is underused as food components, yet has a significant amount of nutrients and excellent in general features. Even though the preliminary findings are very positive, additional effort is needed to develop new products or adjust current systems, in addition to assess the expenses of the procedure and customer perceptions (Lu et al., 2019).

Investigation and improvement concentrating on developing unique and goods with a high level of value, particularly naturopathic remedies and medicines, are also part of upcoming approaches and initiatives for the use of tomato processing residue. Furthermore, compost made from tomato processing residue is seen as a viable organic waste management alternative because of its availability and capacity to build up ground properties and vegetable development. Additional possibility is the bioconversion of tomato processing residue into sustainable renewables that may be either aqueous or vapor, like bioenergy and biofuel. Bioenergy is a potential, inexhaustible, and long-term energy source that can meet growing energy demands while also addressing the depletion of natural gas. Finally, lipase agents are created in solid-state fermentation from tomato processing residue (Lu et al., 2019).

1.5 Chemical composition of tomato processing residue

Peels and seeds make up the majority of tomato processing residue. On a dry matter basis, dietary fibers are the most abundant components in tomato processing waste (25 to 59 %). Overall proteins extend from 15% to 33%, carbs from 3% to 43%, overall lipid from 2% to 20%, and ash from 3% to 6%.

1.6 Carotenoids in tomato processing residue

Carotenoids, which make up around 272 to 554 µg/g of tomato processing residue, are also bioactive compounds (Luengo et al., 2014). Zeaxanthin, lycopene, lutein, cis-β-carotene, and β-

carotene are the primary carotenoids found in tomato processing residue. Tomato processing residue also has a high level of scavenging activity (0.68 to 2.2 mM Trolox/100g). Carotenoids, such as lycopene, are abundant in tomato peels (Knoblich et al., 2005). Because of the high sodium concentration, tomato processing residue cannot be used in animal feed diets (Knoblich et al., 2005).

1.6.1 Lycopene

Lycopene is a lipophilic insoluble compound in water, which is responsible for the characteristic red color of a tomato, watermelon, papaya, pink grapefruit, and pink guava (Figure 1.2). Lycopene considers a hydrocarbon, is sensitive to the effects of heat and is more bioavailable from both processed and cooked tomato products than from fresh tomatoes (Gartner et al., 1997). In the human diet, the consumption of tomato and its derivates constitutes the major source of dietary lycopene compounds (Rao et al., 1998). As a natural antioxidant, this pigment has gotten a lot of interest because of its biological and physiochemical features. Dietary intakes of tomatoes and tomato products consisting of lycopene have been shown in cell culture, animal, and epidemiological investigations to be associated with a decreased risk of chronic diseases such as cancer and cardiovascular disease. Furthermore, serum and tissue lycopene levels have been contrary correlated with the risk of lung and prostate cancers (Heber et al, 2002). In fresh tomatoes, lycopene is mostly found in an all-transform. (Nguyen et al., 2001). Because of the increased energy input, all-trans isomers are synthesized into cis-isomers undergoing isomerization. The bioavailability of lycopene is determined by many factors including isomerization (Nguyen et al., 2001). According to several studies, the cis-isomers of lycopene are better absorbed than the all-trans form. (Boileau et al., 2002). Lycopene concentrations in different types of fruit may depend on several factors including genotype, environmental conditions such as temperature and light,

culture technique management (crop management, fertilizer use, or mineral nutrition and irrigation), as well as fruit and plant phenological stage (growth regulators and ripening stage). Particularly in lycopene, the genotype can prominence the carotenoid content and following discoveries in the medical field about the health benefits of lycopene, some seed companies have received several hybrids to the market, with a lycopene content of up to 188 µg/g (Siviero et al., 2002). Even though, the lycopene production in the tomato fruit is still too low to fully compete with chemical synthesis alternatives (Levin et al., 2004). The highest lycopene content was found in pear types of tomatoes. Pear types of tomatoes contain nearly 500-700 µg/g of lycopene. Tomatoes production in an open field has higher lycopene contents as well as total carotenoids and truss color parameters (tone, brightness, and intensity) than those of fruit produced under protected conditions. In the same way, spring or summer produce has a higher amount than that autumn or winter. Vitamin C content is also suitable to increase the lycopene content according to technical culture practices. Low hydric stress results in the increase in the lycopene content on the tomato truss, as well as that of the ascorbate, generally as a consequence of the reduction in the water content of fruit in stressed plants.

1.6.2 Lycopene biosynthesis in plant cells

Lycopene can be found among the thylakoid membranes in the photosynthetic pigment-protein complex and localized in the chloroplasts of tomato fruits (Bouvier et al., 1998). In the early phases of tomato fruit development, green chlorophyll is the dominant pigment in the chloroplasts. The hue shifts from greenish to white when the chlorophyll deteriorates. Lycopene is biosynthesized with the ultra-structure of the fruit when chlorophyll in the chloroplasts is reduced, resulting in the color shift from white to red. (Harris et al, 1970). The creation of lycopene crystals occurs during the termination of chromoplast development. This lycopene crystal absorbs

a significant amount of chromoplast and appears as voluminous reddish pieces in the chromoplasts (Laval et al, 1974). Lycopene intensities are highest in the peels. (Simpson et al., 1977). Using 14C tracers, researchers have studied the biosynthesis of other carotenoids and lycopene in tomatoes prominently. (Porter et al, 1967). To generate lycopene, mevalonic acid is transformed progressively, with a loss of hydrogen at every level. Each step most likely includes dehydrogenation. Small globules of lycopene form in the chromoplasts, that are immersed in the fruit's whole tomato flesh composition. In the form of crystalline microspheres, lycopene gives tomatoes their distinctively vibrant red color by emitting light.

1.6.3 Physical and chemical properties of lycopene

In ripe tomato fruits, lycopene precedes the form of elongated, needle-like crystals that are bound for the typical bright-red color of ripe tomato fruits. Lycopene is generally soluble in chloroform, benzene, and other organic solvents than in water. Lycopene is an acyclic, open-chain, unsaturated carotenoid having heterocyclic molecule with 13 doubling bonds, 11 of which are linked double bonds arranged in a linear array. Its chemical structure is $C_{40}H_{56}$. The resting methyl groups are at the 1,5-position relative to each other, whereas the two central methyl groups are in the 1,6 positions. A chromatophore with varying lengths was created from a string of ring structure. Lycopene has a special structure that is made up of a radical improvement of ring structure, which gives it its redness and antioxidant effects. Lycopene's widely conjugated polyene structure is what gives it its deep red hue. Seven of these bonds, which are all trans in nature, can isomerize into mono or poly-cis forms under the effects of light, heat, or specific chemical interactions. Lycopene usually appears in an all-trans type. Since lycopene possesses a β-ionone ring structure, it has no pro-vitamin A action. Hill et al. (1969) discovered how chloroplasts transform lycopene to β-carotene. With specific reference to the characteristics of optical emission

in various structural features, isomeric form types of lycopene were differentiated (Zechmeister et al, 1962). Additionally, lycopene is extremely sensitive to heat, light, air, acids that are degrading it, and certain metallic ions such as Cu^{2+}, and Fe^{3+} catalyze its oxidation. Lycopene is one of the natural carotenoids' most potent singlet oxygen quenchers. (Conn et al., 1991). The quenching rate constants (K_q) of different carotenoid species vary significantly. The opening of the β-ionone ring enhances the quenching capacity of lycopene, γ-carotene, and β-carotene, according to a comparison of their structures. The antioxidant capabilities of lycopene and other carotenoids are indicated by their capacity to capture peroxyl radicals and quench singlet oxygen. The quantity of conjugated double bonds in carotenoid species determines their quenching activity, which is influenced to a lesser extent by carotenoid end groups or the type of substituents in carotenoids with cyclic end groups. (Stahl et al., 1993). There are geometric shapes isomers of lycopene, such as poly-cis, all-trans, and mono-cis. The most important geometrical derivative of lycopene in fresh tomatoes is the all-trans isomer which is also the most thermodynamically stable form. During tomato preparation and storage, however, lycopene can undergo trans-to-cis isomerization. The all-trans isoform makes about 35 to 96 % lycopene in varied tomato-based cuisines (Schierle et al., 1996). Using NMR spectroscopy, 15-cis, 9-cis, and 5-cis t subtypes of lycopene in diverse tomato-centered diets and physiological cells were determined. In tomato-based foods, the percentage of 5-cis-isomer ranged from 4 to 27%, whereas the number of other isomers is remarkably lower than 5-cis-isomer (Schierle et al., 1996). In human blood and tissue, the cis-isomers of lycopene account for more than 50% of the total lycopene. (Krinsky et al., 1990). Cis-types have a higher polarity than all-trans isomers and because of their kinked shapes, cis-isomers are less committed to crystallization. The solubility of the cis-isomers in oil and hydrocarbon solvents is also higher than all-trans types. In comparison to all trans-isomers, the performance of

bioactive components of cis-isomers is supplanted by structural morphology modifications. most uniform investigation on lycopene in food systems concern degradation. Lycopene may be partially divested in processed tomato products by heating in the presence of metallic ions (Cu^{2+}, Fe^{3+}, etc.) or oxygen. Lycopene, being a conjugated polyene, is known to encounter at least two transfers, namely, isomerization and degradation, during the preparation of tomatoes,Both in tomato products and pure, lycopene isomerization occurs during processing. The conversion from cis-isomer to trans-form, on the other hand, is a process that could emerge when a commodity is stored. While trans-isomers are in the secure initial state, cis-isomers are in a state that is volatile. Lycopene, as an effective antioxidant, inhibits the formation of extremely reactive singlet oxygen (O_2 ·–) and captures peroxyl radicals (ROO.). Interactions between lycopene and oxygen radicals may be studied as second-order rate reactions. Lycopene is less dynamic and the transmission of an electron can be detected in both directions (Conn et al., 1992). It is also available to form peroxyl radicals capable of acting as a pro-oxidant and undergoes autoxidation. The two primary types of interactions that seem to explain oxygen functionalities are the formation to a carbon-carbon double bonding and the displacement of a methyl or methylene group. At both ends of the typical C40-carbon framework, in particular, oxidative breakdown is possible. Lycopene is insoluble in water and solubility in oil at 20 °C ranged between 70-80 µg/g and the vegetable oils with extracted lycopene could be used for the creation of functional food with natural lycopene.

1.6.4 Health benefits of lycopene

Lycopene is a powerful antioxidant, which has lots of health benefits. Antioxidants are very important for many reasons, especially in a world where processed food has eradicated most of what gives the body the ability to prevent and fight disease. Lycopene is an antioxidant that might as well be worth its weight in gold for the incredible things from which it

protects our body. Consumption of a diet rich in tomatoes has been corresponding with decreased risk for several chronic diseases, including heart disease and cancer, specifically prostate cancer, and this decreased risk is often associated with lycopene Processed tomato products are a worldwide commodity that yielded over 210 million ton in 2012 (FAOSTAT, 2012). For an adult, recommended daily intake of lycopene is 5-10 mg/day. Lycopene has been conversely related to the risk of prostate cancer in studies conducted in North America (Giovannucci et al, 1999), as well as to other cancer events in several studies from North America, Europe, China, and Japan (Giovannucci et al, 1999). Lycopene can regulate the pathogenesis of cancer and cardiovascular disease.

1.6.5 Extraction of lycopene

Food processing by-products are increasingly being used as a source of functional food components. Lycopene is typically stable in tomato matrices following modest heat treatments, according to the literature. However, degradation and isomerization may occur quickly under intensive processing conditions or when lycopene is dissolved in oil or chemical solvents (Colle et al., 2010). The extraction of lycopene can be done in several different ways.

1.6.5.1 Solvent extraction

The most used technique for lycopene extraction is solvent extraction. But using organic hazardous solvents like n-hexane, acetone, ethyl acetate and ethanol comes with a number of issues, including toxic effects, solvent residue in the final result, and management difficulties (Eh and Teoh 2012).

1.6.5.2 Ultrasound-assisted extraction

Ultrasound is an effective extraction technique that encourages mass transfer by pretending on the unit to destroy the membrane and cell matrix. It may cause a variety of effects in a solvent, including hydroxyl electrons, the heat effect, and bubbling (Kumcuoglu, Yilmaz, & Tavman, 2014). UAE was recognized as a cost-effective, efficient in terms of time, resource, and chemicals extraction process. Similar to organic solvents, such as ethanol, hexane and acetone, commonly assisted in extracting lycopene. The effects followed the order: ethyl ether < ethyl acetate < <hexane $<CH_2C_{l2}$ (Wu, Shen, Yin, & Cheng, 2017). Yasmini, Silva, Ferreira, Celli, and Brooks (2018) were able to extract lycopene (1330 µg/g) using UAE from tomatoby products with ethyl acetate: ethyl lactate (3:7) (7:3), which is comparatively higher than organic solvent by 10%. Although plant-based oil did not affect lycopene removal, it improved bio-accessibility (Anese, Bot, Panozzo, Mirolo, & Lippe, 2015). Polymer electrolytes and polar aprotic solvents were also popular in the Ultrasound-assisted extraction. Significantly, due to their superior qualities, they may be able to eradicate the detrimental impacts of organic solvents on the environment. (Martins & de Rosso, 2016) With sufficient intervals, pulsed ultrasound is more efficient than continuous ultrasonication due to the absorption and dispersion of the ultrasound wave caused by the ongoing formation of dewatering droplets in the steady state. UAE's ultrasonic power and duration were consistently between 10-50 min and 50–200 W, approximately indicating that it was a viable approach for lycopene extraction when compared to SE.

1.6.5.3 Microwave-assisted extraction

In microwave-assisted extraction, microwave radiation has the potential to destroy or inactivate the cell wall. Microwave-induced molecular resonance the cell of vegetation might cause in a local maximum heat, which could compromise cell integrity and encourage cellular

chemical transfer. 135.9 µg/g of trans-type lycopene was obtained from tomato by Ho, Ferruzzi, Liceaga, and San Martín-Gonz´alez (2015) at 24 kJ, 400 W, 1:20 g/mL, counterparts for 60 s with ethyl acetate, contrasted to the ~40 µg/g for 30 min of organic solvent extraction. Moreover, whereas the removal procedures did not influence the cis-lycopene content, they had a considerable impact on the cis/trans proportions and overall lycopene amount. With SE or microwave processing, Honda et al. (2018) obtained varying the amount of lycopene in dehydrated gac aril. Microwave pretreatment significantly boosted lycopene concentration in the press and ethanol groups at 20°C, while it decreased at 4°C in the class of acetone among all extractions, the lycopene content, as well as Z-isomers, was greatest at 1 min and 1050 W using pressure removals.

1.6.5.4 Supercritical fluid extraction

This extraction methods were widely used in lycopene removal from a variety of vegetations, together with tomatoes, guava, pitanga pulp, and watermelon because of the effective and sustainable liquid (Zuknik, Nik Norulaini, & Mohd Omar, 2012). Because solubility altered fast near the critical point of supercritical fluid, SFE may liquefy or expel the electrolyte quickly. SFE's moderate temperature was typically favorable for the preservation of lycopene (the minimum range of CO_2, for instance, was 31 °C). The majority of studies utilized CO_2 as a diluent and fine-tuned removal constraints such as heat, pressure, fluid stream speed and duration. Lycopene extraction can be done in a number of ways including from tomato processing by-products (1 L/min, 50 MPa, 80 ˚C and 0.358 µg/g, (Hatamib, Angela, Meirelesb, & N. Ciftcia, 2019), Furthermore, modifiers for instance dichloromethane, hexane, acetone, methanol and ethanol , might have a substantial impact on the process, with acetone behaving the best. Vegetable oils that are co-solvents, such as canola oil and hazelnut oil may increase lycopene improvement

and permanence due to their lipid solubility (Bruno et al., 2018). Due to polarity and matrix alteration, the solubility altered with, force, heat and the addition of ethanol or oil as a co-solvent.

1.6.5.5 Chemical method - production with microemulsion

The lipophilic/hydrophilic chemicals in the solvent might be taken up via microemulsion, which could produce stable dispersions. Surfactants and co-surfactant were often employed to improve implementation by lowering the atomic interaction of light (Amiri-Rigi & Abbasi, 2016). The microemulsion process was influenced differentially by the emulsifier lecithin, Tween 20, sucrose monopalmitate span 20, saponin, Tween 80, Tween 60, rhamnolipid, and rhamnolipid, with saponin performing best, SMP second, and lecithin last; also, Co-surfactants may have an impact on the procedure (ethanol with one hydroxyl group, propylene glycol with two, and glycerol with three, correspondingly),although not the same as strongly as emulsifier. (Amiri-Rigi & Abbasi, 2016, 2017). To increase efficiency, microemulsion employed environmentally friendly emulsifier rather than natural solvents, avoiding environmental risk (Kirkhus et al., 2019). More crucially, because the ability of emulsions might be modified with adding emulsifiers and co-emulsifiers, it was readily increased and managed. Furthermore, lower interfacial tension might increase the solubility of lipophilic compounds in fluid, lowering the need for organic solvents.

1.7 Emulsions

Emulsions typically contain a minimum of one insoluble liquid that is disseminated into a different liquid in the form of small droplets with the aid of an emulsifier. Emulsion droplets display all the classical behaviors of metastable colloids: Brownian motion, reversible phase transitions as a result of droplet interactions that may be completely adjusted, and irreversible transitions that generally contain their destruction. They are received by shearing two immiscible fluids to accelerate the fragmentation of one phase into the other. As a result, the lifetime of

emulsions may become important (more than a year), and they become good candidates for various commercial applications. double emulsions. A colloidal is a more generic category of different systems of substance that includes emulsions. Although the words colloidal and emulsion are occasionally used in opposition to one another, emulsion is the preferred term when the scattered and continuous phases are both fluids. Emulsifier is a term used to describe a material that equalizes an emulsion. Emulsion is well-known for its various application. Nowadays, emulsion is used to extract bioactive compounds. This procedure is regarded as an environment friendly extraction approach. Because the system has both hydrophilic and lipophilic regions, it may incorporate many bioactive substances (Roohinejad et al. 2014). Additionally, a significant portion of water, a protic solvent that can both contribute and absorb hydrogen bonds, is present in the oil-in-water (O/W) emulsion. This activity could contribute to tomato cell wall expansion and lycopene release. Three solvent characteristics—hydrogen bonding capability, basity and molar volume —have the most effects on the process of cell enlargement (Mantanis et al.1994; Prusov et al. 2014). Additionally, a prior study revealed that the solvent's protic or aprotic character affects how the plant matrix expand (Fidale et al. 2008). This innovative extraction technique may contribute to lowering the amount of artificial and dangerous ingredients used in food, medicine, and beauty products while also improving their reliability and increasing consumer acceptance.

1.8 Objectives of this research

The general objective of this research is to the extraction of lycopene from tomato processing residue using O/W emulsion. In addition, the study was specifically aimed to:

- To investigate the lycopene recovery from tomato processing residue using organic solvents, triglycerides, fatty acids, and oil-in-water emulsions with or without of emulsifier and its antioxidant activity
- To investigate the effects of different extraction conditions on lycopene recovery and antioxidant activity
- To investigate the chemical stability of lycopene and antioxidant

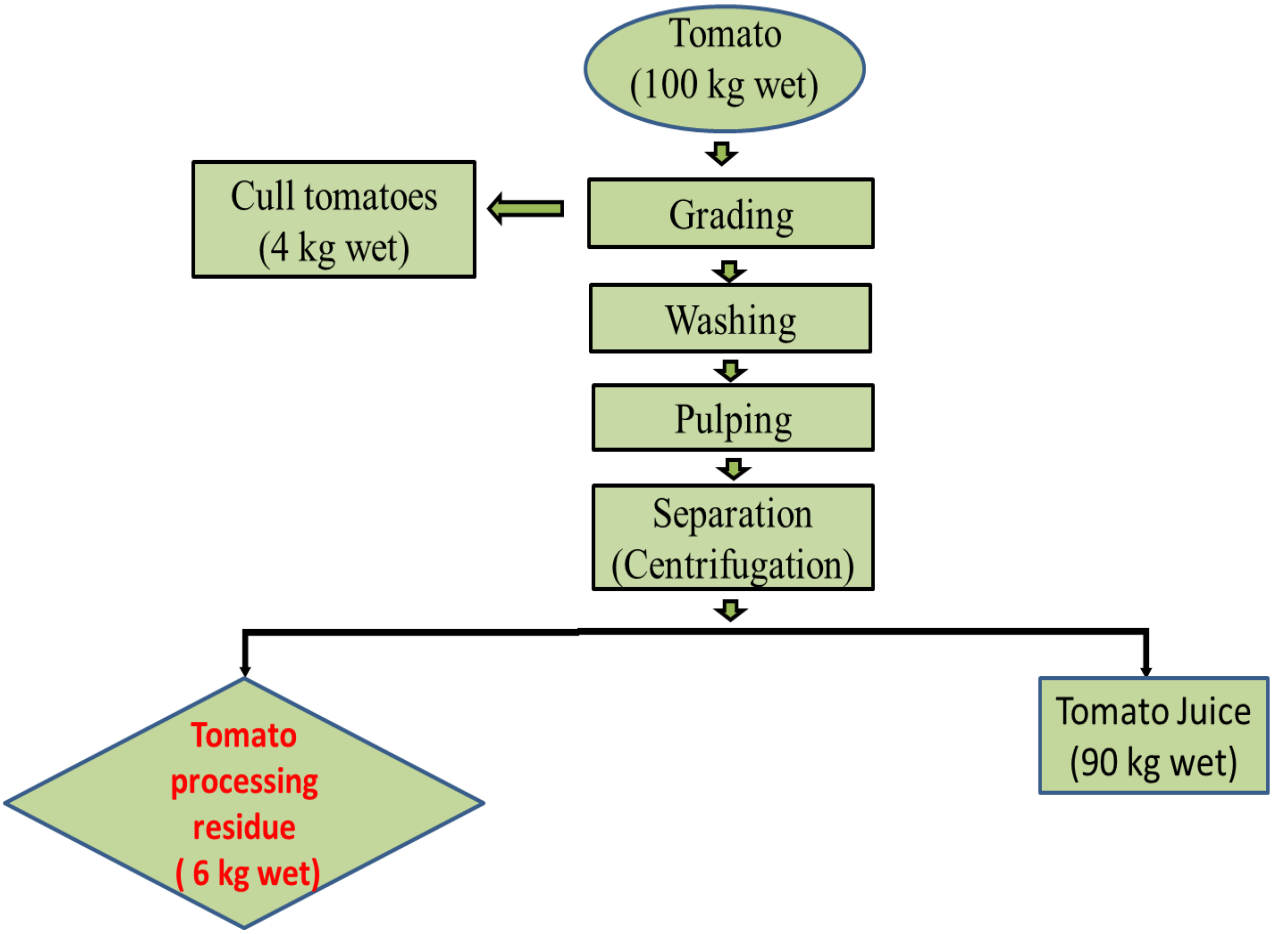


Figure 1.1: Flow diagram of the tomato processing

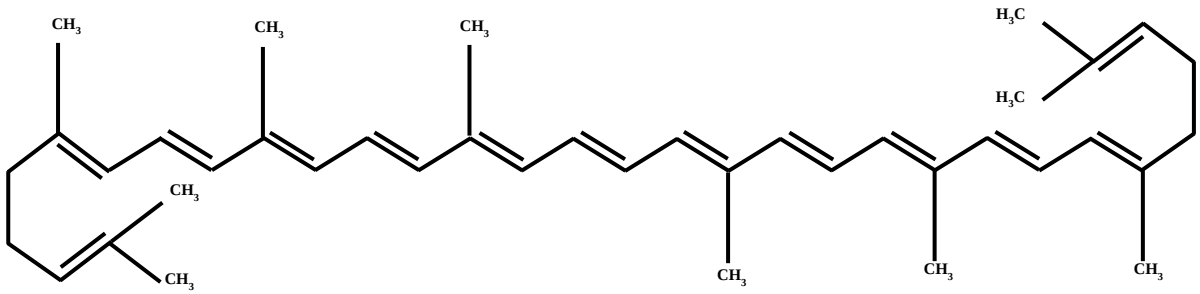


Figure 1.2: Chemical structure of lycopene

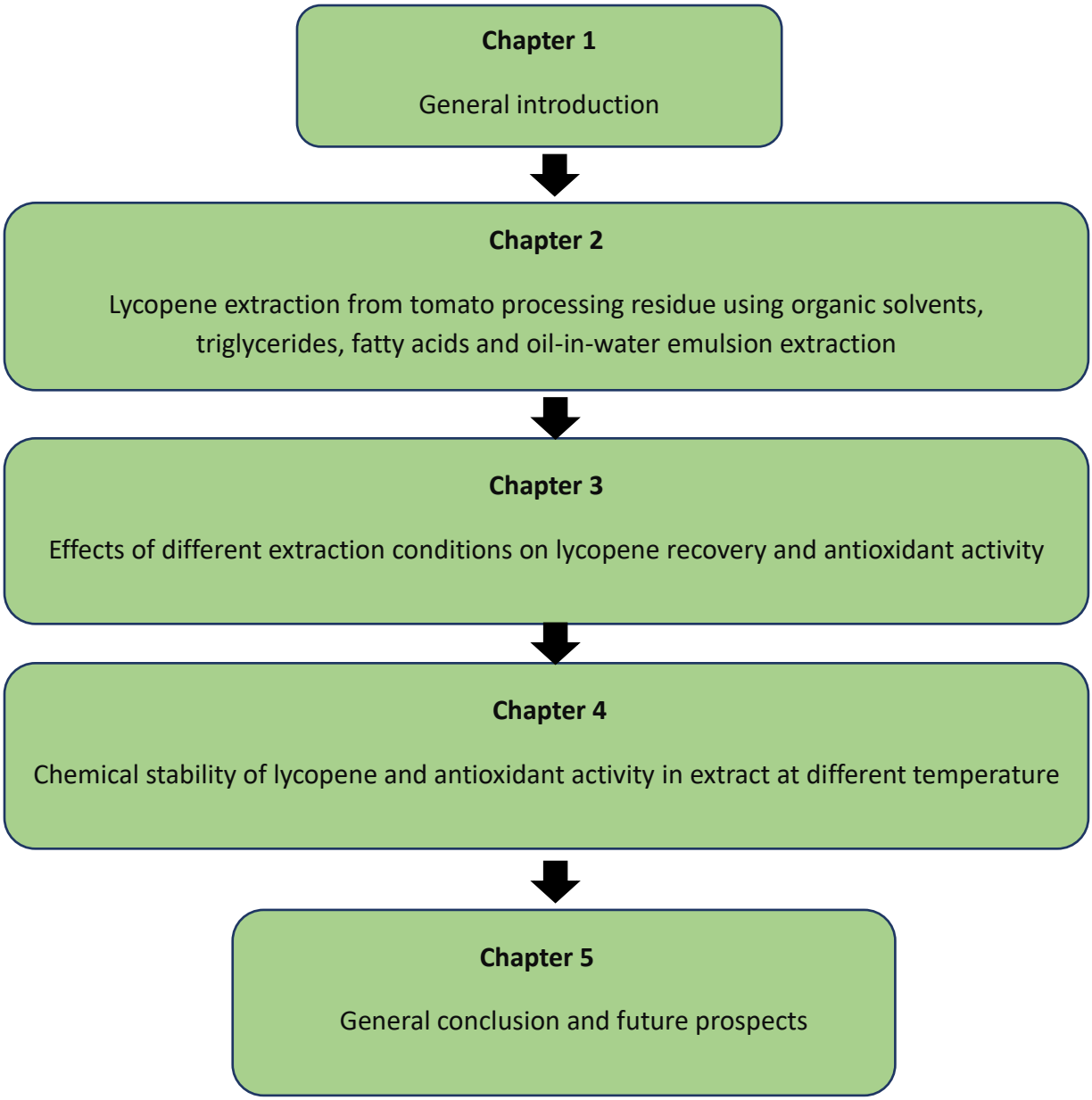


Figure 1.3: Outlines of the thesis

Chapter 2

Lycopene extraction from tomato processing residue using organic solvents, triglycerides, fatty acids and oil-in-water emulsion

2.1 Introduction

Lycopene ($C_{40}H_{56}$) is a carotenoid that has hydrocarbon with 13 carbon-carbon double bonds, 11 of which are linearly oriented. Lycopene has a remarkable antioxidant impact due to its high degree of conjugation, creating it among the greatest effective radical scavengers. (Zuorro, 2020). It is very efficient in oxygen singlet quench ($^1O_2$), a powerful oxidizing molecule that may damage lipids, proteins, and nucleic acids, among other biological components. (Amiri-Rigi and Abbasi, 2016). It has twice the power to quench singlet oxygen, according to prior studies, and is tenfold greater than the concentrations of α-tocopherol and β-carotene, correspondingly. (Deng et al., 2021). Furthermore, disease related research shows that it may aid in the prevention of brain diseases, diabetes, cancers and heart disease including Parkinson's, and Huntington's disease (Li et al., 2021; Zuorro, 2020). Because it is a potent radical scavenger lycopene assists in the decrease of oxidative distresses which are commonly linked to the development of the disorders mentioned. Furthermore, additional defense mechanisms than oxidative stress, such as lycopene's ability to limit cell growth, directly damage, and improve spacing intercellular connection between cells, might be closely associated. The aforementioned findings have raised demand for carotenoid-based skin care, therapeutic meals, and remedies. Thus, the lycopene industries have expanded significantly both in scale and quality (Deng et al., 2021).

Lycopene is prevalent in tomato as well as other red vegetables and fruits(Li et al., 2021). The majority of the organic lycopene is derived from tomato pericarp and by-products. Tomatoes are among highest sources of natural antioxidants, and they are frequently recognized as a dietary supplement with many physiological features that assist to reduce the incidence of cancers such as lung, prostate, stomach, and prostate (Eh and Teoh, 2012). Vitamin E, ascorbic acid, phenolic acids, flavonoids, and carotenoids are among the antioxidants found in them. Lycopene is a

carotenoid that gives tomatoes their rich red color. (Amiri-Rigi and Abbasi 2016). Many physical separation techniques of lycopene have previously been devised. Almost every method is economically unviable for extensive manufacturing. Solvent extraction is the most popular technique for lycopene extraction. Consequently, using volatile chemical liquids like ethyl acetate, acetone, , n-hexane, and ethanol, has quite a lot of problems, together with poisoning, solvent residues in the completed project, as well as dumping issues. (Eh and Teoh, 2012). There are some other lycopene extraction approaches, such as, microwave-based extraction, ultrasound-based extraction, enzyme-based extraction and supercritical fluid extraction. Nonetheless, current separation technologies are highly costly, take a lengthy period to complete, and require a lot of momentum (Deng et al., 2021).

Microemulsions have been used to extract a variety of bioactive chemicals during the last several years. For example, a lecithin-based microemulsion technology was used to extract canola oil from the seed of the canola plant (Abbasi and Radi 2016). Using a microemulsion method, lycopene and β-carotene were recovered from tomato and carrot by-product separately (Amiri-Rigi and Abbasi, 2019)

Emulsions are made up a minimum of one immiscible fluid that is spread inside the form of a drop to the other with the help of an emulsifier. This process is referred to as a "green" extraction method. Because the arrangement has both hydrophilic and lipophilic regions, it may incorporate a variety of bioactive compounds (Roohinejad et al., 2014). Furthermore, water, a protic liquid that is able to contribute and absorb hydrogen bonds, is present in large quantities in (O/W) emulsions. This activity might help the tomato tissue membrane enlarge and promote carotenoid restoration. Three solvent factors impact the process of cellular swelling: molar volume, basicity,

and hydrogen bonding ability. Furthermore, a prior report found that the solvent's aprotic or protic character affects the plant matrix's swelling process.

Even though utilizing artificial surfactants in microemulsion-assisted separation improves overall extraction efficiency by raising osmotic pressure, which causes cell expansion, there are rising safety concerns about its usage at elevated intensities in cuisine and decorative presentations. In the absence of a synthetic emulsifier, however, no reports of lycopene O/W emulsion-assisted extraction have been identified. This unique extraction method might aid in the reduction of synthetic and dangerous compounds in food, pharmaceutical, and cosmetic applications, as well as improve their safety and client acceptance.

Lauric acid is a green solvent that is well-recognized for the ability to separate carotenoid substances effectively. With a reduced viscosity, greater polarity, lower molar volume, stronger hydrogen bond capacity and greater polarity, it has a greater inflammation quality. Lauric acid has the benefit of increasing total extraction efficiency due to these exceptional features (Table 2.1). Lauric acid is also a fatty acid nutritive enhancement that may provide nutritional benefits. It’s important mentioning that no previous research on the impacts of Lauric acid-based O/W emulsions in lycopene recovery has been done to our knowledge. Moreover, the hypotheses are:

(1) The emulsion system increased the surface area, which may help to enhance lycopene recovery

(2) Protic solvent, water helps to enlarge the tissue membrane of the plant which may help during extraction (Prusov et al., 2014).

Therefore, the aim of this research is to investigate the lycopene recovery from tomato processing residue using organic solvents, triglycerides, fatty acids, and oil-in-water emulsions with or without of emulsifier and its antioxidant activity

2.2 Materials and Methods

2.2.1 Materials

Dried tomato processing residue was provided by Pran Food Ltd, Ekdala, Natore, Bangladesh. Acetone, hexane, ethanol (99.5%), and ethanol (99.5%) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka Japan). A Milli-Q system (Sartorius, Arium® pro, Goettingen, Germany) was used to produce deionized water (18 MΩ cm).

2.2.2 Preparation of tomato processing residue powder

Dried tomato processing residue with a moisture content of approximately 7.04% wt was ground using (Ultra Centrifugal Mill ZM 200, Retsch GmbH & Co. KG) into a homogeneous powder with a particle size of 500 µm and stored at -20 ˚C until further analysis.

2.2.3 Organic solvent extraction

Tomato processing residue (2 g) was combined with 30 mL of acetone, ethanol (99.5%), and hexane and agitated at 25 ° C for 3 h. The solid particles were then removed from the suspensions by centrifuging them at 9,100 x g for 1 hour. Finally, a filter (PTFE - 0.45 um) and analysis of the supernatant were performed.

2.2.4 Fatty acids and triglycerides-based extraction

Tomato processing residue (2 g) was combined with 30 ml of soybean oil, MCT oil, tributyrin, caproic acid, caprylic acid, capric acid, lauric acid, or oleic acid, and mixing using magnetic stirrers (3 h, 50°C). The filtrates were examined to determine their lycopene concentration after the particles had been eliminated.

2.2.5 Fatty acids and triglycerides-based Oil-in-water emulsion extraction

Oil-in-water emulsion-assisted extraction was determined by Tsogtoo et al., (2020) with certain adjustments. In order to prepare oil-in-water emulsions, 30 mL of the triglyceride or fatty acid phase (soybean oil, MCT oil, tributyrin, caproic acid, caprylic acid, capric acid, lauric acid, or oleic acid) were mixed with 70 mL of the aqueous phase, either with or without the addition of 0.1 % (w/w) saponin emulsifier. The coarse emulsions were formed in a rotor-stator homogenizer for 5 minutes at 7000 rpm (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland). The formed emulsions (100 mL) were then mixed, along with 2 g of powdered tomato processing residue, and swirled for 3 hours at 50 ° C at 750 rpm. The supernatants were examined to determine their lycopene concentration after the solid particles had been removed.

2.2.6 Lycopene measurement

With minor adjustments from Fish et al. (2002), the overall lycopene concentration in the tomato processing residue was examined. In a summary, 3 mL of Milli-Q water was added to the solution and stirred for an additional 5 minutes after 2 g of tomato processing residue powder was extracted with a 20 mL fresh combination of acetone, hexane and ethanol, (1:2:1) under continuous stirring (700 rpm) for 15 min at 25 °C. The mixture was then let to spontaneously split into polar and nonpolar phases by resting at 25°C for 5 minutes. The upper phase was filtered through a hydrophobic membrane (0.45 m) and the absorbance was assessed at 503 nm using a UV spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The bottom phase was re-extracted until it left a whitish residue. The lycopene content of the tomato processing residue was calculated following Equation (1):

$$Lycopene\ content\ \left(\frac{\mu g}{g}\right) = \frac{A_{503} \times 31.2 \times Dilution}{Amount\ of\ sample} \quad (1)$$

Where A_{503} and 31.2 are the absorbance of the extract at 503 nm and a constant.

2.2.7 Lycopene recovery

The recovery of lycopene extraction R (%) from tomato processing residue was established applying Equation (2):

$$R\ (\%) = C/C_t \times 100 \quad (2)$$

Where C is the lycopene in tomato processing residue (µg/g), C_t is the total lycopene in tomato processing residue (µg/g). In this study, 588.06 µg/g of total lycopene content in tomato processing residue was investigated

2.2.8 Antioxidant activity

According to DPPH free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl) (Górnaś et al., 2014; Shen et al., 2010), the antioxidant activity assessment was accomplished with slight adjustments. In a 3 mL ethyl acetate solution (0.1 mM in ethyl acetate), 1 mL extracts of the given solvent extract were added. After that, the mixture was placed in a dark room for 30 minutes. 1 mL ethyl acetate in 3 mL DPPH radical solution was used to make a blank solution. In order to quantify the absorbance, a UV spectrophotometer was used at 517 nm (V-530, Jasco Corporation, Tokyo, Japan). Each sample was measured three times. The following equation was used to represent the results in terms of radical scavenging activity:

$$\text{Radical scavenging activity} = A_{control}\text{-}A_{sample}/A_{control} \times 100 \quad (3)$$

2.2.9 Droplet size measurement

Using a static laser diffraction particle size analyzer, the volume mean diameter (d4,3) of the soybean oil emulsion with or without an emulsifier was assessed (LS 13,320, Beckman Coulter, Brea, USA). Water and soybean oil both had refractive indexes of 1.33 and 1.47, respectively.

Equation (4) defined the volume mean diameter (d4,3) as the average droplet diameter:

$$d_{4,3}=\sum n_i d_i^{\,4} / \sum n_i d_i^{\,2} \qquad (4)$$

where, d_i = diameter, and n_i = the overall number of droplets that are d_i in diameter. d_i.

2.2.10 Statistical analysis

Each experiment is carried out in at least three duplicates, and the mean and standard deviation of the findings are presented in this research. Using Statistic 8.1 software, the analysis of variance (ANOVA) was used to compare the lycopene content in various conditions at a 95 % confidence level ($p < 0.05$). (Tallahassee, USA).

2.3 Results and discussion

2.3.1 Effects of organic solvent extraction on lycopene recovery

Figure 2.1 shows the extraction efficiency of the lycopene using organic solvents. Lycopene extraction efficiency was approximately 37% of tomato processing residue using hexane. As shown in figure 2.2: a, the low polarity value of hexane helps to enhance the lycopene recovery.

In contrast, ethanol resulted in a substantially reduced recovery 25.5 % (Figure 2.1). This lower extraction efficiency can be described by the low high polarity value of ethanol.

In extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques. The energy required to produce room in the molecules for other molecules is determined using Hildebrand's standard solubility parameter (Hansen 2007). As a result, the role of chemical attraction in maximizing recoveries were estimated by comparing the solubility characteristics of lycopene and the solvents. It is well recognized that the closer two components' solubility parameters are closed, the stronger their attraction is. On the contrary, the solvent's particle mass also has an impact on Hildebrand solubility.

The dissolving parameter of an atom (δ) is equivalent to the repulsive energy content doubled, according to Hildebrand and Scott's classic solubility hypothesis. As a result, it can be states as:

$$\delta = \sqrt{\frac{\Delta E}{v_m}} \tag{5}$$

Where ΔE is the the repulsive energy content, and v_m is the the molecular volume, respectively.

The overall repulsive energy, according to Hansen, may be separated into three segments: hydrogen-bonding, polarity and dispersion (Hansen 2007). Therefore, the measure of overall dissolution may be written as:

$$\delta^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (6)$$

where δ_D , δ_P, and δ_H are the dispersion parameter, polar parameter, and hydrogen-bonding solubility parameter, respectively.

Furthermore, the space (D) in Hansen solubility can be used to indicate the attraction of a solute (A) for a diluent (B). This distance may be calculated by adding the points together corresponding to the solute ($\delta_{D,A}$, $\delta_{P,A}$, $\delta_{H,A}$) and the diluent ($\delta_{D,B}$, $\delta_{P,B}$, $\delta_{H,B}$), as follows:

$$D = \sqrt{(\delta_{D,A} - \delta_{D,B})^2 + (\delta_{P,A} - \delta_{P,B})^2 (\delta_{H,A} - \delta_{H,B})^2} \quad (7)$$

The chemical connections between A and B can be illustrated by length, according to equation 7. The molecular interactions are comparable with a tiny D-value, indicating strong attraction.

Table 2.1 displays the D-values and solubility parameters for lycopene and the solvents. According to equation 6, Distance can demonstrate the chemical bonding between A and B. The interaction forces are comparable and show good attachment with a modest D-value. (Figure 2.2: b). The solubility characteristics and D-values for lycopene and solvent are shown in Table 2.1. The affinity for lycopene of ethanol, acetone, and hexane can be established as follows: ethanol<acetone<hexane, respectively. The most efficient organic solvent, hexane, for lycopene absorption exhibited comparatively low distance (D = 0.7 MPa $^{0.5}$) (Table 2.1). Therefore, the attraction of lycopene for hexane is higher than other diluents. Thus, hexane showed the highest recovery of lycopene.

2.3.2 Effects of triglyceride-based extraction on lycopene recovery

Figure 2.3 demonstrates the extraction efficiency of the lycopene using triglyceride-based extraction. Lycopene extraction efficiency was approximately 52% of tomato processing residue using tributyrin.

In the contrast, soybean oil resulted in a lower recovery of approximately 44 % (Figure 2.4: a). This lower extraction efficiency can be described by the high viscosity of soybean oil.

As explained in the previous section, in extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques. Table 2.2 illustrates the D-values and solubility parameters for lycopene and the liquids. Tributyrin with the maximum D-values for lycopene recovery (D = 6.94 MPa $^{0.5}$) was shown to be the most effective (Table 2.2). As a result, lycopene has a lower affinity for tributyrin than other solvents (Figure 2.4: b). A previous study looked into this inconsistency and found that the most efficient lycopene solvent combination had a rather high distance (Zuorro 2020). Thus, variables apart from dissolved affinity might have an influence on the procedure. Hansen's dissolving sphere includes a low-distance solvent, but it cannot absorb the content, according to prior research. This might be because of the molecule’s huge dimensions, which makes it difficult for it to permeate the solute (Yamamoto et al. 2017). Furthermore, even if their solubility parameters are the same, Hildebrand's solubility parameter theory predicts that smaller molar volume solvents are preferred to bigger molar volume solvents. As a result, the size of the solvent molecule might be an important 4th factor in this solubility estimates the foregoing concerns hint at a possible justification for the O/W emulsion-assisted extraction techniques' observed outcomes. Tributyrin consist of a modest length of atoms when contrasted to other

triglycerides. Tributyrin's small molecular size allows it to permeate the plant matrix, increasing lycopene recovery.

2.3.3 Effects of fatty acid-based extraction on lycopene recovery

Figure 2.5 demonstrates the recovery of the lycopene using fatty acids. Lycopene extraction efficiency was almost 57% of tomato processing residue using lauric acid. In contrast, oleic acid resulted in a roughly reduced recovery 49.2 % (Figure 2.6: a).

As explained before, in extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques. The energy required to produce room in the molecules for other molecules is determined using Hildebrand's standard solubility parameter (Hansen 2007). As a result, the role of liquid attraction in optimizing recovery was estimated by comparing the solubility characteristics of lycopene and the solvents. It is well recognized that the closer two components' solubility parameters are closed, the stronger their affinity is.

The molecular interactions are comparable with a tiny D-value, indicating strong attraction.

Table 2.2 provides the dissolution factors and D-values for lycopene and the liquids. According to equation 7, distance can demonstrate the chemical bonding between A and B. The interactions are comparable with a tiny D-value, indicating great connection. The dissolution characteristics and distance parameter for lycopene and solvent are shown in Table 2.2. The affinity for lycopene of caproic acid, caprylic acid, lauric acid, or oleic acid can be established as follows: oleic acid<caproic acid<caprylic acid<capric acid<lauric acid, respectively. One of the best fatty acid, lauric acid, for lycopene extraction, indicated comparatively low D-values (D =

8.43 MPa $^{0.5}$) (Table 2.2). Thus, the attraction of lycopene for lauric acid is higher than other molecules (Figure 2.6). Thus, lauric acid showed the highest recovery of lycopene.

2.3.4 Effects of triglyceride-based O/W emulsion (without emulsifier) extraction on lycopene recovery

Figure 2.7 shows the recovery of lycopene using O/W emulsion-assisted extraction without an emulsifier. Without surfactant, the lycopene recovery was almost 45% using soybean oil-based O/W emulsion.

In contrast, tributyrin-based O/W emulsion without surfactant resulted in a roughly lower restoration 35 %.

As explained in the previous section, in extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques. The energy required to produce room in the molecules for other molecules is determined using Hildebrand's standard solubility parameter (Hansen 2007). As a result, the significance of liquid affinity in improving recovery was estimated by comparing the solubility characteristics of lycopene and the solvents. It is well recognized that the closer two components' solubility parameters are closed, the stronger their affinity is.

For O/W emulsion, the following equation can be used to determine the solubility parameter:

$$\delta_{O/W\ emulsion} = \sum_i \varphi_i\, \delta_i \qquad (8)$$

where φ_i and δ_i be a symbol of the ith component's percentage in the O/W emulsion and its Hansen absorption factor, respectively.

Furthermore, the space (D) in Hansen parameter can be used to illustrate the solvent's (B) affinity for a molecule (A) This distance may be calculated by adding the points together corresponding to the molecule ($\delta_{D,A}$, $\delta_{P,A}$, $\delta_{H,A}$) and the solvent ($\delta_{D,B}$, $\delta_{P,B}$, $\delta_{H,B}$), as follows:

$$D = \sqrt{(\delta_{D,A} - \delta_{D,B})^2 + (\delta_{P,A} - \delta_{P,B})^2 (\delta_{H,A} - \delta_{H,B})^2} \quad (9)$$

The chemical connections between B and A can be illustrated by Hansen space, according to equation 9. The molecular interactions are comparable with a tiny D-value, indicating strong affinity.

The molecular interactions are comparable with a tiny D-value, indicating good attachment. Table 2.3 displays the solvent's D-values and the lycopene's solubility properties. The affinity for lycopene of soybean oil, MCT oil, or tributyrin O/W emulsion can be established as follows: soybean oil >MCT oil>tributyrin O/W emulsion without an emulsifier, respectively. The most efficient solvent, soybean oil O/W emulsion without an emulsifier, for lycopene absorption demonstrated considerably lower D-values (D = 32.63 MPa $^{0.5}$) (Table 2.3). As a result, the affinity of lycopene for soybean oil O/W emulsion is higher than other O/W emulsions (Figure 2.8). Thus, soybean oil O/W emulsion without an emulsifier showed the highest recovery of lycopene.

2.3.5 Effects of fatty acid-based O/W emulsion (without emulsifier) extraction on lycopene recovery

Figure 2.9 shows lycopene recovery using fatty acids-based O/W emulsion-assisted extraction without an emulsifier. Without saponin, the lycopene recovery was almost 73.1% using lauric acid-based oil-in-water emulsion.

In the contrast, oleic acid-based O/W emulsion without saponin lead to a substantially lower recovery 33 %.

As explained in the previous section, in extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques The energy required to produce room in the molecules for other molecules is determined using Hildebrand's standard solubility parameter (Hansen 2007). As a result, the significance of liquid affinity in improving recovery was estimated by comparing the solubility characteristics of lycopene and the solvents. It is well recognized that the closer two components' solubility parameters are closed, the stronger their affinity is. The molecular interactions are comparable with a tiny D-value, indicating strong affinity.

According to equation 9, length can illustrate the interactions between A and B. The interactions are comparable with a tiny D-value, indicating great connection. Table 2.3 displays the solvent's D-values and the lycopene's solubility properties. The affinity for lycopene of caproic acid, caprylic acid, lauric acid, or oleic acid can be established as follows: oleic acid<caproic acid<caprylic acid<capric acid<lauric acid O/W emulsion without an emulsifier, respectively. The most efficient lauric acid O/W emulsion, for lycopene absorption demonstrated considerably lower D-values (D = 34.1 MPa $^{0.5}$) (Figure 2.10). As a result, the attraction of lycopene for lauric acid O/W emulsion is higher than other O/W emulsions. Thus, lauric acid oil-in-water emulsion showed the highest recovery.

2.3.6 Effects of triglyceride-based O/W emulsion (with emulsifier) extraction on lycopene recovery

Figure 2.11 reveals the extraction efficiency of lycopene using O/W emulsion-based extraction with an emulsifier. With saponin, the lycopene recovery was around 26% using Tributyrin-based O/W emulsion.

In contrast, soybean oil-based O/W emulsion with emulsifier caused a recovery reduction of around 22%.

As explained in the previous section, in extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques. Table 2.3 displays the Hansen space and solubility characteristics for lycopene and the liquids. Tributyrin O/W emulsion with emulsifier with the maximum D-values for lycopene recovery (D = 33.6 MPa $^{0.5}$) was shown to be the most effective (Figure 2.12: b). As a result, lycopene has a lower affinity for tributyrin O/W emulsion than other solvents emulsions. A previous study looked into this inconsistency and found that the most efficient lycopene solvent combination had a rather high Hansen space (Zuorro 2020). Thus, characteristics besides liquid affinity may have an impact on the procedure. Hansen's dissolving spherical includes a short-range solvent, but it does not absorb the solute. according to prior research. This might be because of the solvent's huge size, which makes it difficult for it to permeate the solute (Yamamoto et al. 2017). Furthermore, even if their solubility parameters are the same, Hildebrand's solubility parameter theory predicts that smaller molar volume solvents are preferred to bigger molar volume solvents. As a result, the size of the solvent molecule might be an important 4th factor in Hansen solubility estimates the foregoing consequences hint at a possible

clarification for the O/W emulsion-assisted extraction techniques' observed outcomes. Tributyrin has a modest atom’s size when correlated to other triglycerides. Tributyrin's small molecular size with low viscosity (figure 2.12: a) allows it to permeate the plant matrix, increasing lycopene recovery.

2.3.7 Effects of fatty acid-based O/W emulsion (with emulsifier) extraction on lycopene recovery

Figure 2.13 shows lycopene recovery using fatty acids-based O/W emulsion-assisted extraction without an emulsifier. With saponin the lycopene recovery was almost 30.2% using lauric acid-based O/W emulsion.

In contrast, oleic acid-based O/W emulsion with emulsifier caused a recovery reduction of around 21.4 %.

As explained in the previous section, in extraction systems, for absorption, Hildebrand solubility is frequently accustomed to assessing the efficacy of diluents in equally traditional and oil-in-water emulsion extraction techniques. The energy required to produce room in the molecules for other molecules is determined using Hildebrand's standard solubility parameter (Hansen 2007). As a result, the importance of liquid affinity in enhancing recovery was estimated by comparing the solubility characteristics of lycopene and the solvents. It is well recognized that the closer two components' solubility parameters are closed, the stronger their affinity. The molecular interactions are comparable with a tiny D-value, indicating strong affinity.

According to equation 9, Length can illustrate the reaction mechanism between A and B. The interaction forces are comparable with a tiny D-value, indicating particular fondness. Table

2.3 provides the solubility properties and D-values of lycopene and solvents the affinity for lycopene of caproic acid, caprylic acid, lauric acid, or oleic acid can be established as follows: oleic acid<caproic acid<caprylic acid<capric acid<lauric acid O/W emulsion without an emulsifier, respectively. The most efficient lauric acid O/W emulsion demonstrated considerably lower D-values for the absorption of lycopene (D = 34.14 MPa $^{0.5}$) (Table 2.3). Therefore, the attraction of lycopene for lauric acid O/W emulsion is higher than other O/W emulsions (Figure 2.14). Thus, lauric acid oil-in-water emulsion showed the highest extraction efficiency.

2.3.8 Possible mechanism of O/W emulsion extraction with or without an emulsifier.

As shown in Figure 2.15, lauric acid-based oil-in-water emulsion resulted in the highest lycopene recovery 73.1%. In contrast, lauric acid-based oil-in-water emulsion with natural emulsifier saponin reflected in a substantially lower recovery. 30.2%. The inability to interact between lycopene and triglyceride or fatty acid droplets in the O/W emulsion extraction technique may account for the reduced extraction efficiency. An emulsifier forms a barrier across the solvent when triglyceride or fatty acid droplets are coated with it, which lessens the interaction between the solute and the powder. (Tsogtoo et al. 2020).

Furthermore, the solvent's swelling ability can be used to explain the previously described highest recovery. Cellulose is structured as microfibrils in cellulosic material, with both amorphous and crystalline areas. Slightly thicker fibers made of microfibrils are subsequently cross-linked by hemicelluloses and encased in a cellulosic matrix that simulates a jelly. (Lavecchia and Zuorro 2016). The percentage of cellulose content and the spatial arrangement of the lignocellulosic materials network are regulated by intermolecular and intramolecular interaction. These links are produced by the 4-linked D-glucopyranose units of cellulose-1 that contain hydroxyl groups.

(Grunin et al. 2015). Solvent molecules can penetrate the plant matrix and adsorb to these hydroxyl groups due to their small size and strong polarity. As a result of adsorption, the substance expands as a function of connections that are disrupted, which widen the distance between lignocellulosic strands (Prusov et al. 2014). The amorphous portions of cellulosic, that are less reactive and liquid exposed, are the only places where expansion may occur. As previously mentioned, the capacity of the solvent to form hydrogen bonds, its basicity, and its molar volume all have an impact on the swelling process. The swelling process is also influenced by the solvent's protic or aprotic character (Fidale et al. 2008). because hydrogen bonds may be given and taken by protic solvents like water.

As hypothesized in the introduction section, the existence of a large volume of water, swelling behavior during O/W emulsion extraction is greatly amplified and emulsion enhances the surface area by small droplets size (Figure 2.16) For the lauric acid-based oil-in-water emulsion without an emulsifier, the addition of protic solvent water is thought to allow the tomato processing residue tissue to enlarge. This enlargement helps lauric acid permeate the vegetal tissue, boosting the efficiency of lycopene extraction (73.1%).

Recovering of lycopene seemed less when an emulsifier was used to create the lauric acid-based oil-in-water emulsion than when it wasn't. There is a possibility that a lauric acid-based oil-in-water emulsion with 0.1% (w/w) natural emulsifier saponin will be less able to expand the vegetal tissue, however it was observed that the emulsion's hydrogen bond capability was not noticeably affected by the addition of this little quantity of emulsifier (Table 2.3). Consequently, the decreased capacity of lycopene to access the fatty acids and triglycerides droplets' core might be the cause of the saponin emulsifier's lessened capability to recover lycopene from the particles' surface (Figure: 2.15). (Amiri-Rigi and Abbasi 2016).

The principle of lycopene extraction from the O/W emulsion may be summed up as follows: A) the solvent's tiny molar mass aids in permeating the plant matrix, facilitating lycopene extraction; and B) the existence of a significant amount of protic solvent (water), and promotes the plant’s structure to enlarge by generating hydrogen-bond complexes with the atoms of the cell wall.

2.3.9 Antioxidant activity

Scrounging the stable DPPH radical approach is a common approach for determining antioxidant activity. DPPH is a stable free radical with a 517 nm absorption wavelength and antioxidants react with DPPH and convert it to 2.2-diphenyl-1-picrylhydrazine. The degree of discoloration indicates the antioxidant extract's scavenging power, which is attributable to its hydrogen donating ability (Van Gadow et al., 1997). DPPH radical-scavenging abilities of the tomato processing residue extracts are shown in Figure 2.17 (c) soybean oil-based O/W emulsion without emulsifier showed high scavenging activity was 95%.

2.4 Conclusion

Using fatty acids, triglycerides, organic solvents, and O/W emulsion-assisted extraction, lycopene was recovered from tomato processing residue in this investigation. Comparing O/W emulsion to organic solvents, triglycerides, or fatty acids, we found that it was more effective in extracting lycopene. Without emulsifier system, the O/W emulsion's capacity to expand aids in enlarging the cell wall, which accelerates recovery.

Table 2.1 Hansen solubility parameters and molecular properties of organic solvents and lycopene.

Compound	δ_D (MPa $^{0.5}$)	δ_P (MPa $^{0.5}$)	δ_H (MPa $^{0.5}$)	δ (MPa $^{0.5}$)	D (MPa $^{0.5}$)
Ethanol	15.8	8.8	19.4	26.52	21.30
Acetone	15.5	10.4	7	19.94	12.54
Hexane	14.9	0	0	14.90	0.70
Lycopene	15.6	0	0	15.60	-

δ_D, dispersion ; δ_P, polar , δ_H,hydrogen bond , δ, total Hansen solubility ; D, distance between points in Hansen space.

Table 2.2 Triglycerides, fatty acids, and lycopene solubility characteristics.

Compound	δ_D (MPa $^{0.5}$)	δ_P (MPa $^{0.5}$)	δ_H (MPa $^{0.5}$)	δ (MPa $^{0.5}$)	D (MPa $^{0.5}$)
Soybean oil	16.5	2.0	2.7	16.80	3.47
Mct oil	3.6	2.8	4.5	6.41	13.12
Tributyrin	16.3[1]	3.9[1]	5.7[1]	17.70[1]	6.94
Caproic acid	16.1	5.5	11.1	20.31	12.40
Caprylic acid	16.1	5.1	9.5	19.38	10.79
Capric acid	16.2	4.2	8.3	18.68	9.32
Lauric acid	16.2	4	7.4	18.25	8.43
Oleic acid	14.3	3.1	14.3	20.46	14.69
Saponin	20	23.23	14.3	33.82	27.63
Lycopene	15.6	0	0	15.60	-

δ_D, dispersion ; δ_P, polar , δ_H,hydrogen bond , δ, total Hansen solubility ; D, distance between points in Hansen space.

Table 2.3 Triglyceride and fatty acid-based O/W emulsion solubility characteristics with or without of 0.1% (w/w) saponin emulsifier.

Types of O/W emulsion	Compound	δ_D (MPa $^{0.5}$)	δ_P (MPa $^{0.5}$)	δ_H (MPa $^{0.5}$)	δ (MPa $^{0.5}$)	D (MPa $^{0.5}$)
Without emulsifier	**Soybean oil**	15.80	11.80	30.42	36.25	32.63
	Mct oil	11.93	12.04	30.96	35.30	33.42
	Tributyrin	15.74	12.37	31.32	37.17	33.67
	Caproic acid	15.68	12.85	32.94	38.68	35.36
	Caprylic acid	15.68	12.73	32.46	38.23	34.87
	Capric acid	15.71	12.46	32.1	37.85	34.43
	Lauric acid	15.71	12.4	31.83	37.60	34.16
	Oleic acid	15.14	12.13	33.90	39.06	36.01
With emulsifier	**Soybean oil**	**15.80**	**11.81**	**30.40**	**36.24**	**32.61**
	Mct oil	**11.93**	**12.05**	**30.94**	**35.28**	**33.40**
	Tributyrin	**15.74**	**12.38**	**31.30**	**37.16**	**33.66**
	Caproic acid	**15.68**	**12.86**	**32.92**	**38.66**	**35.34**
	Caprylic acid	**11.93**	**12.74**	**32.44**	**36.84**	**35.04**
	Capric acid	**10.94**	**12.47**	**32.08**	**36.11**	**34.73**
	Lauric acid	**15.71**	**12.41**	**31.81**	**37.59**	**34.14**
	Oleic acid	15.14	12.13	33.88	39.04	35.99

δ_D,dispersion ; δ_P,polar , δ_H, hydrogen bond , δ, total Hansen solubility ; D, distance between points in Hansen space.

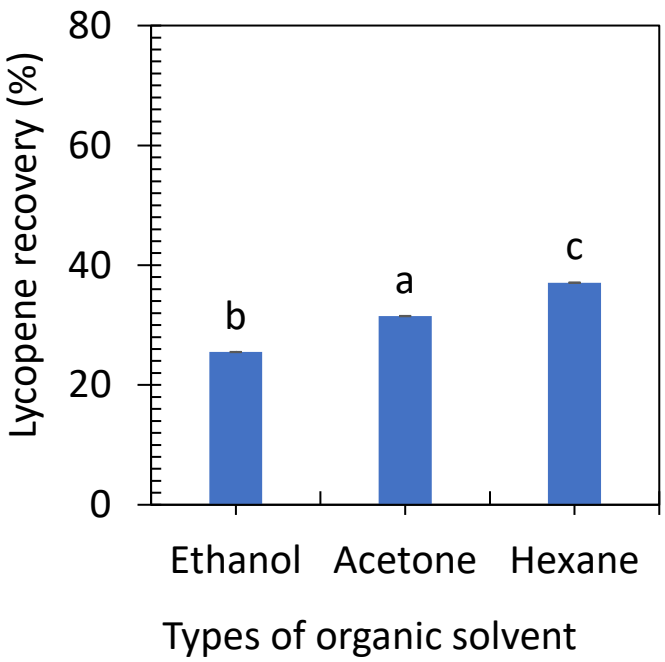
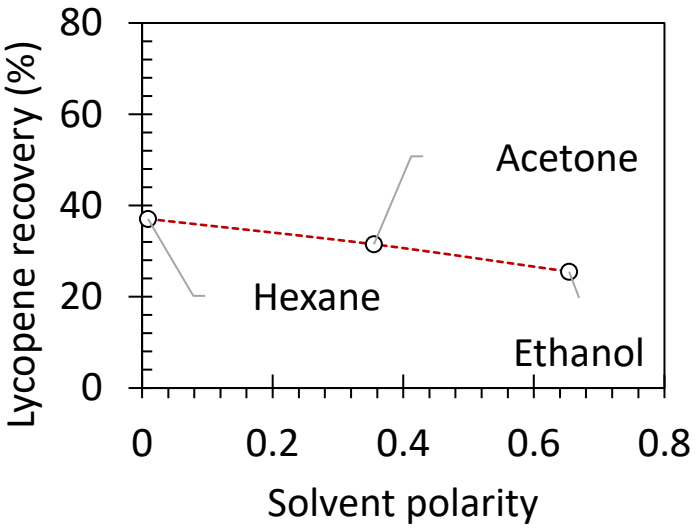


Figure 2.1: Extraction of lycopene from tomato processing residue using organic solvent extraction.

(a)



(b)

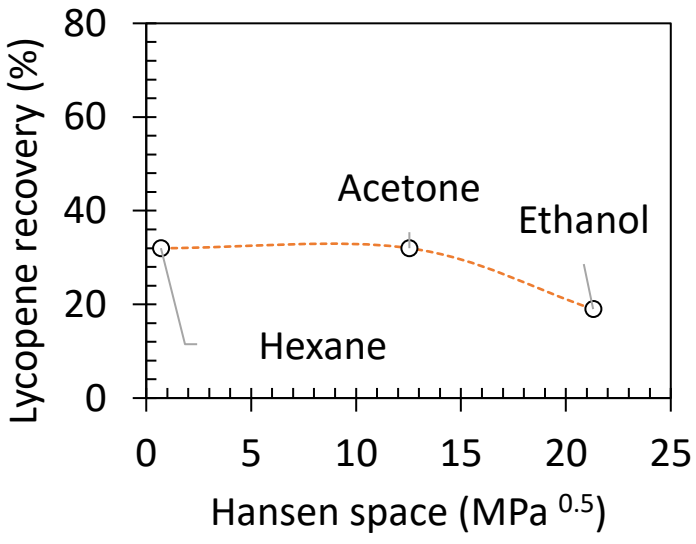


Figure 2.2: Effects of (a) solvent polarity and (b) Hansen space on lycopene recovery from tomato processing residue.

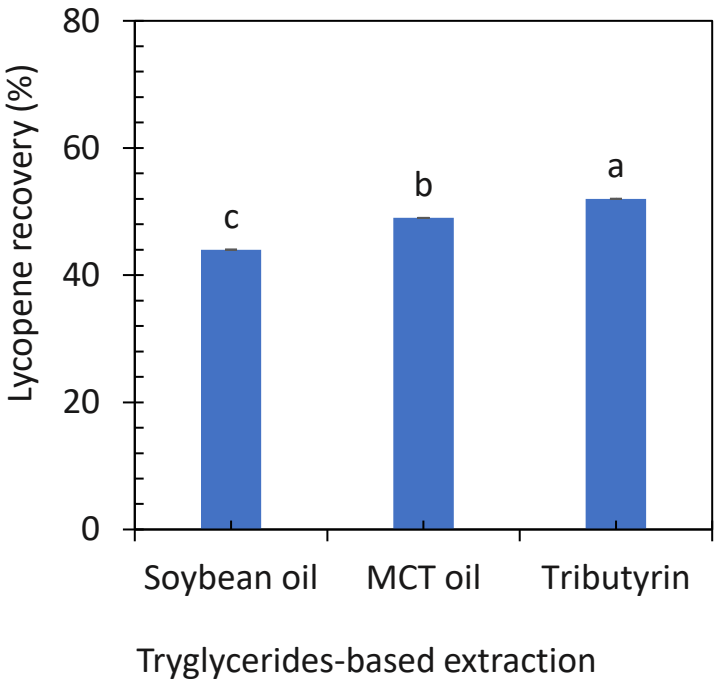
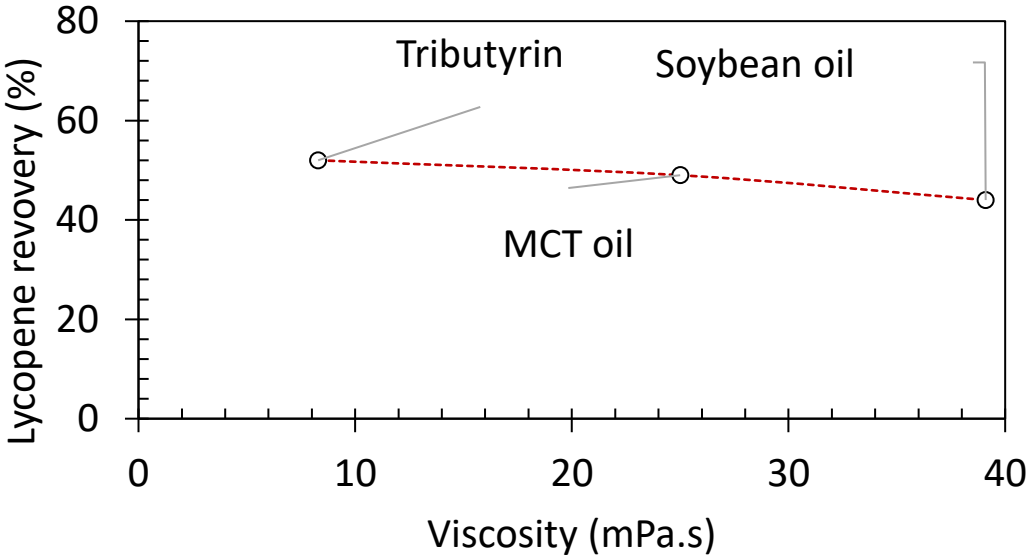


Figure 2.3: Extraction of lycopene from tomato processing residue using different triglycerides.

(a)



(b)

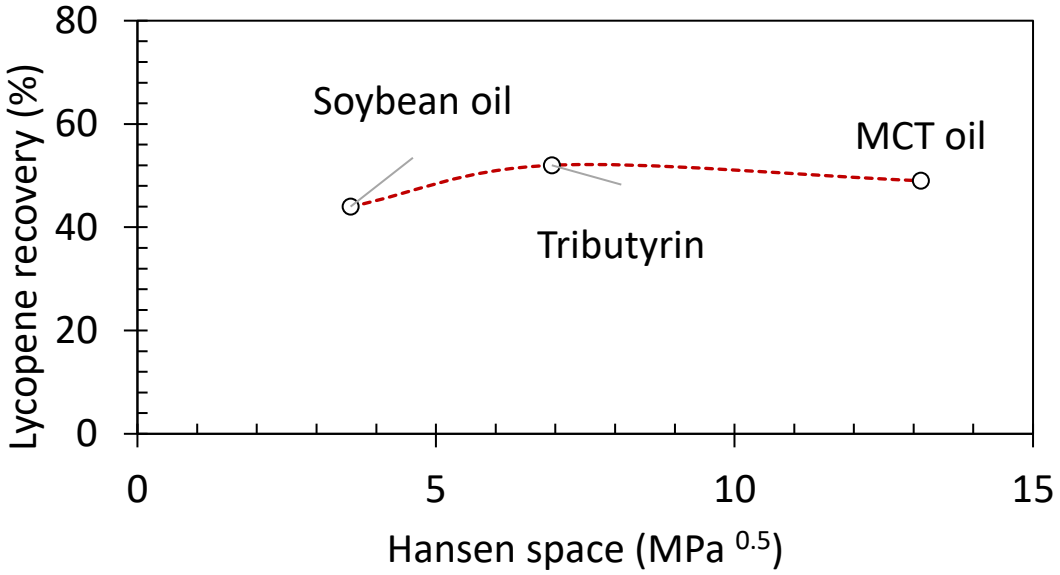


Figure 2.4: Effects of (a) viscosity and (b) Hansen space on lycopene recovery from tomato processing residue.

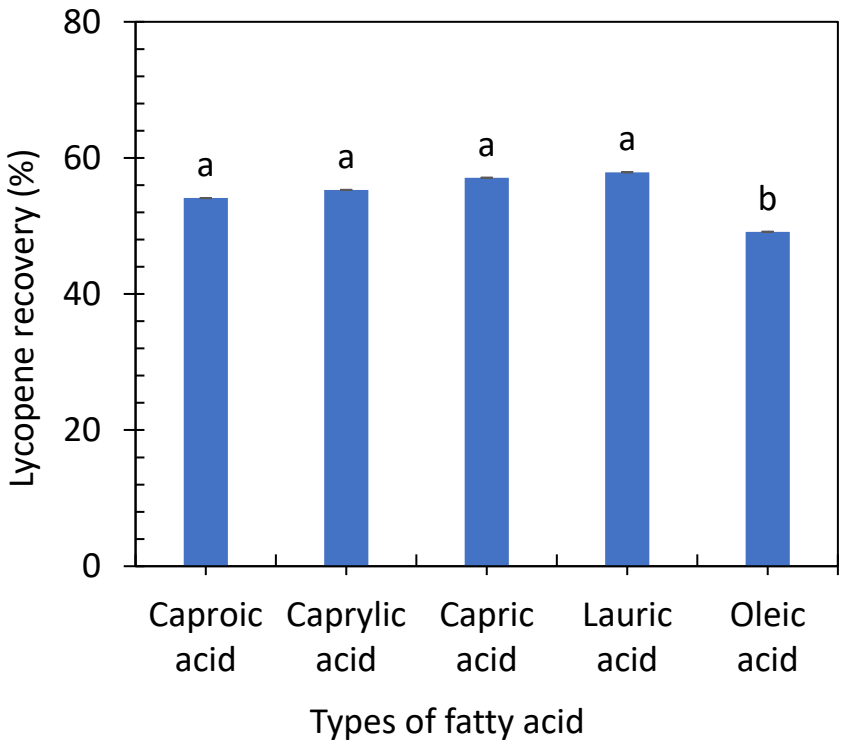


Figure 2.5: Extraction of lycopene from tomato processing residue using different fatty acids.

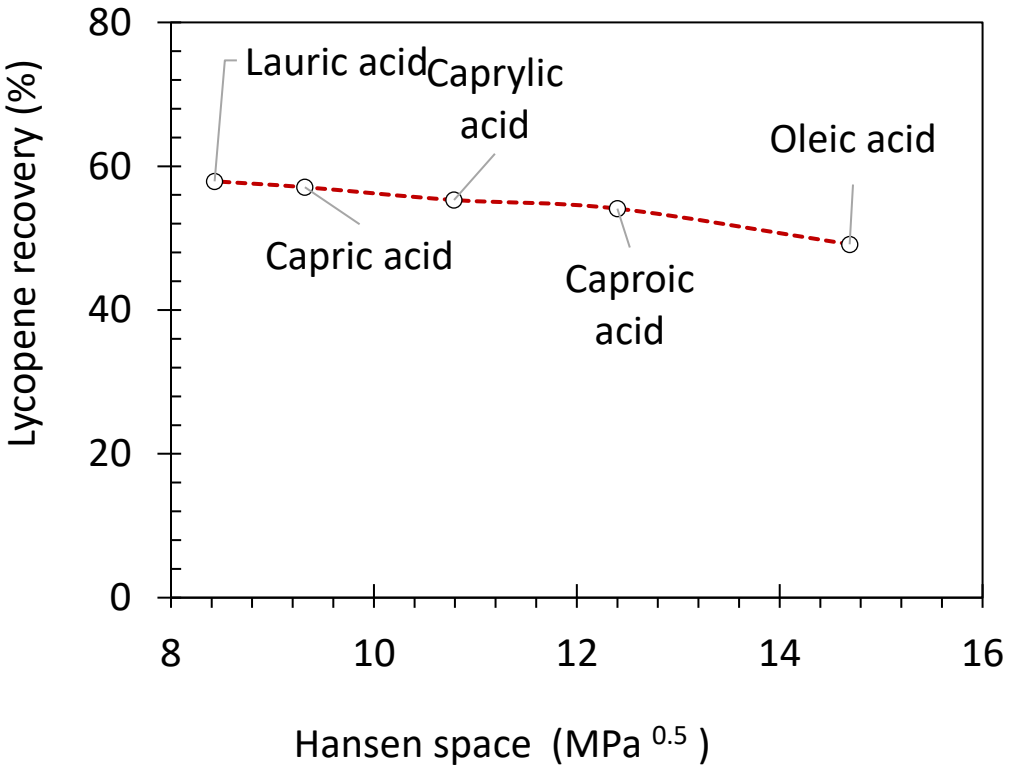


Figure 2.6: Effects of Hansen space on lycopene recovery from tomato processing residue.

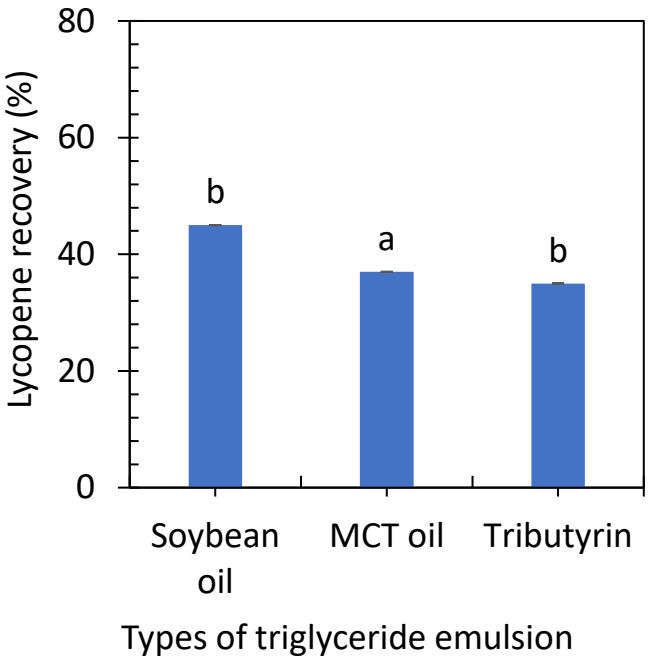


Figure 2.7: Extraction of lycopene from tomato processing residue using different triglycerides-based O/W emulsion extraction without emulsifier.

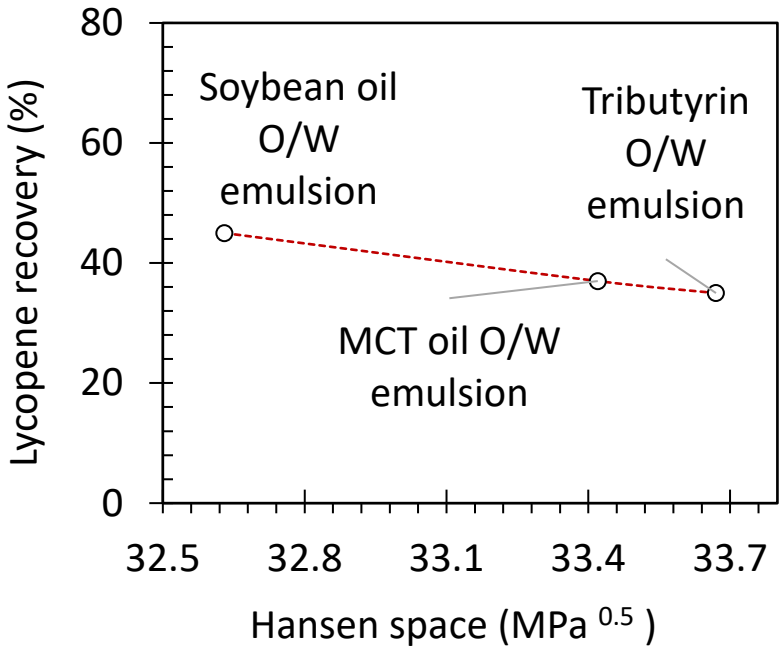


Figure 2.8: Effects of Hansen space on lycopene recovery from tomato processing residue.

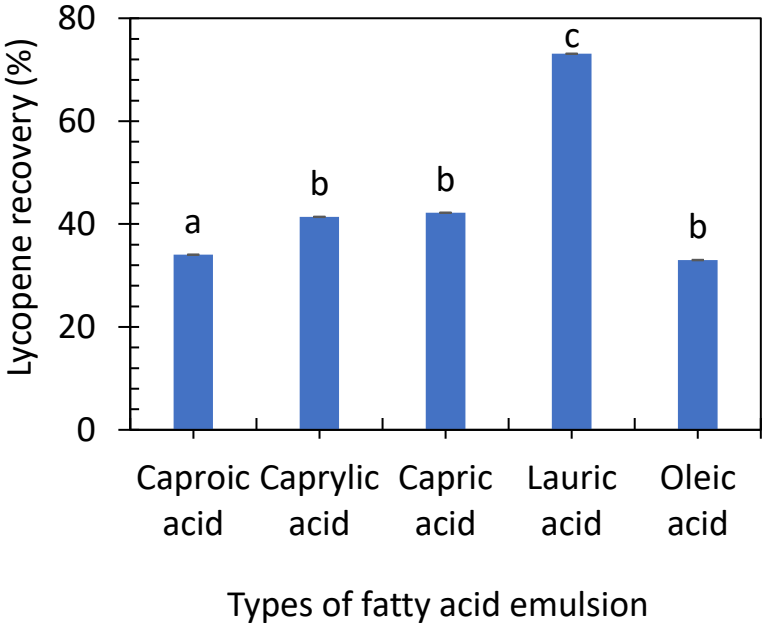


Figure 2.9: Extraction of lycopene from tomato processing residue using different fatty acids-based O/W emulsion extraction without emulsifier.

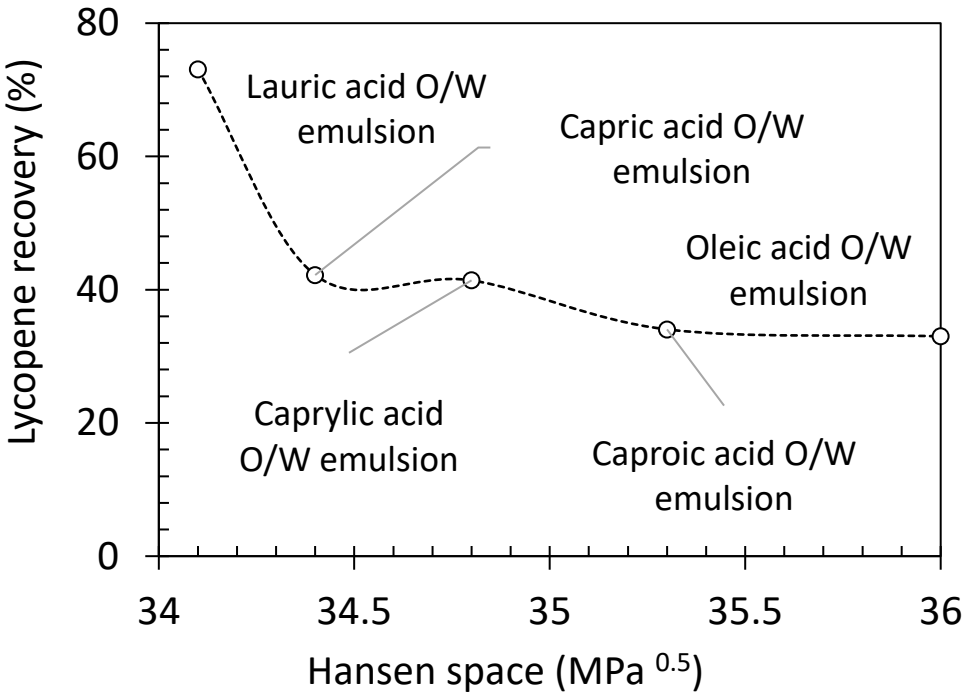


Figure 2.10: Effects of Hansen space on lycopene recovery from tomato processing residue.

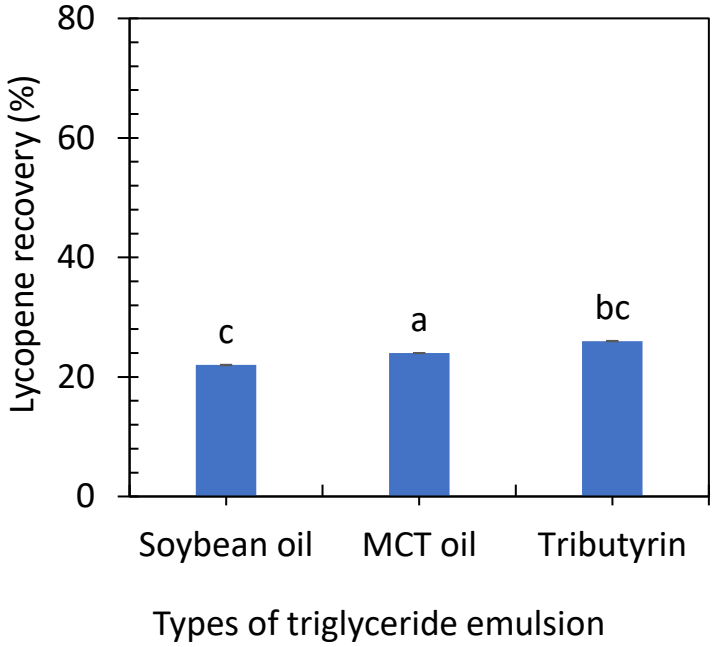
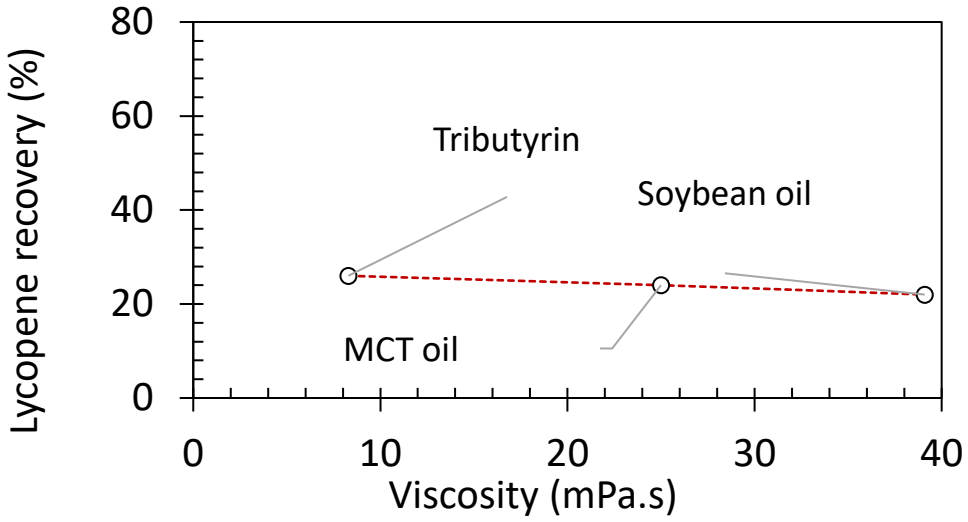


Figure 2.11: Extraction of lycopene from tomato processing residue using different triglycerides-based O/W emulsion extraction with emulsifier.

(a)



(b)

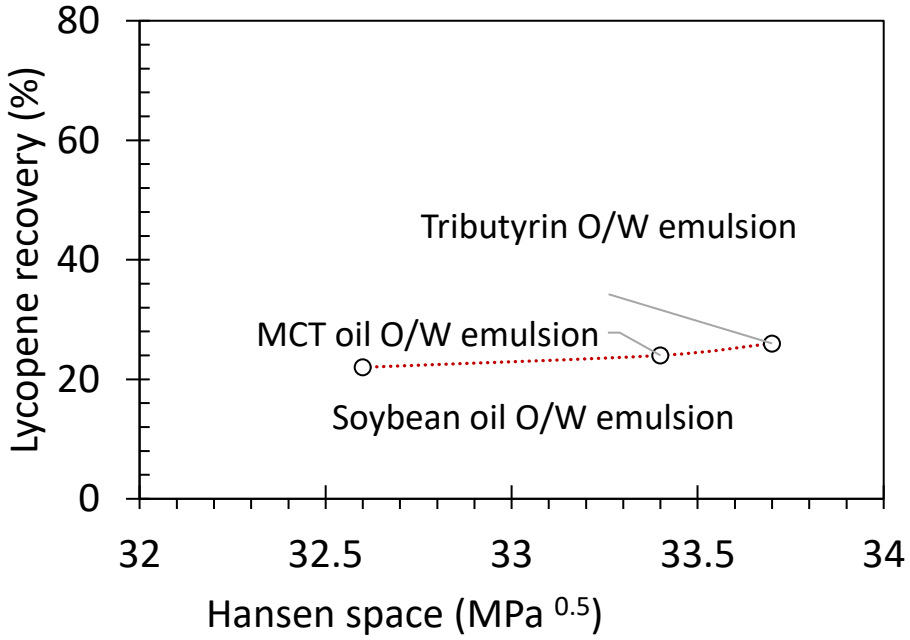


Figure 2.12: Effects of (a) Viscosity and (b) Hansen space on lycopene recovery from tomato processing residue.

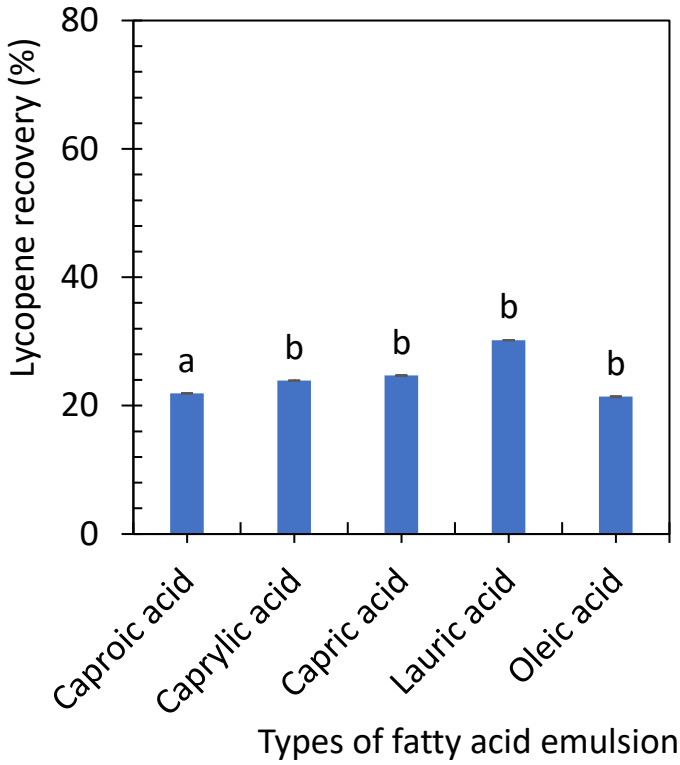


Figure 2.13: Extraction of lycopene from tomato processing residue using different fatty acids-based O/W emulsion extraction with emulsifier.

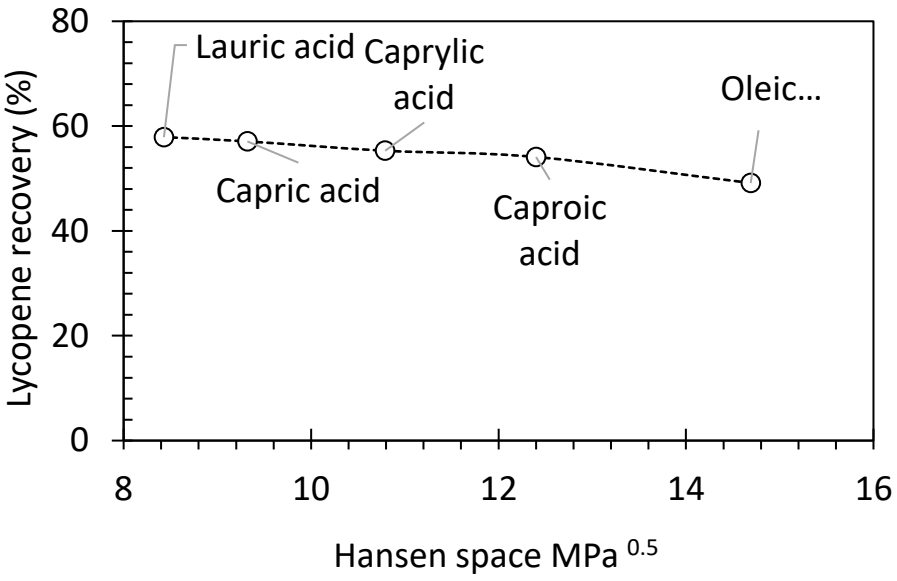


Figure 2.14: Effects of Hansen space on lycopene recovery from tomato processing residue

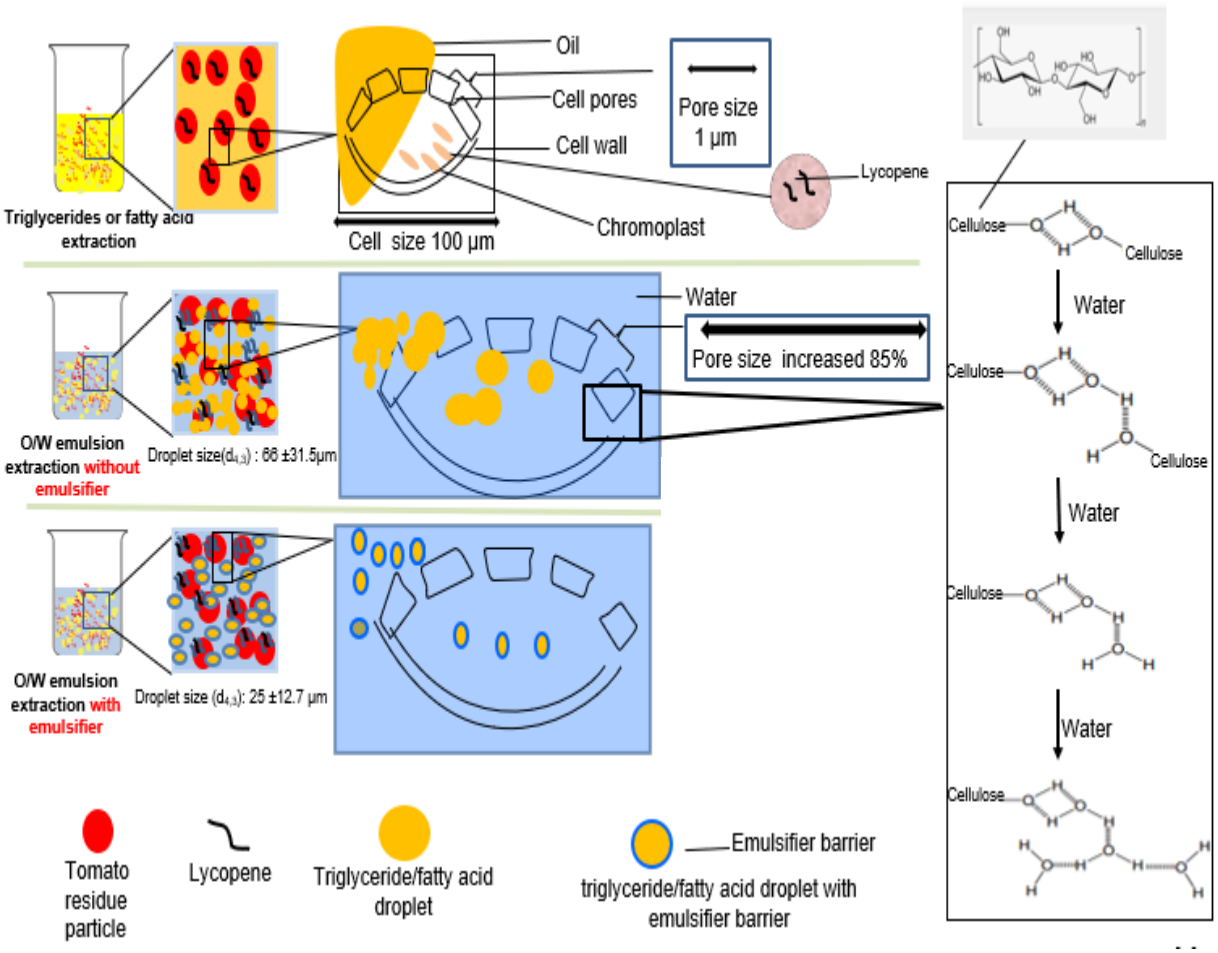


Figure 2.15: Possible mechanism of O/W emulsion extraction

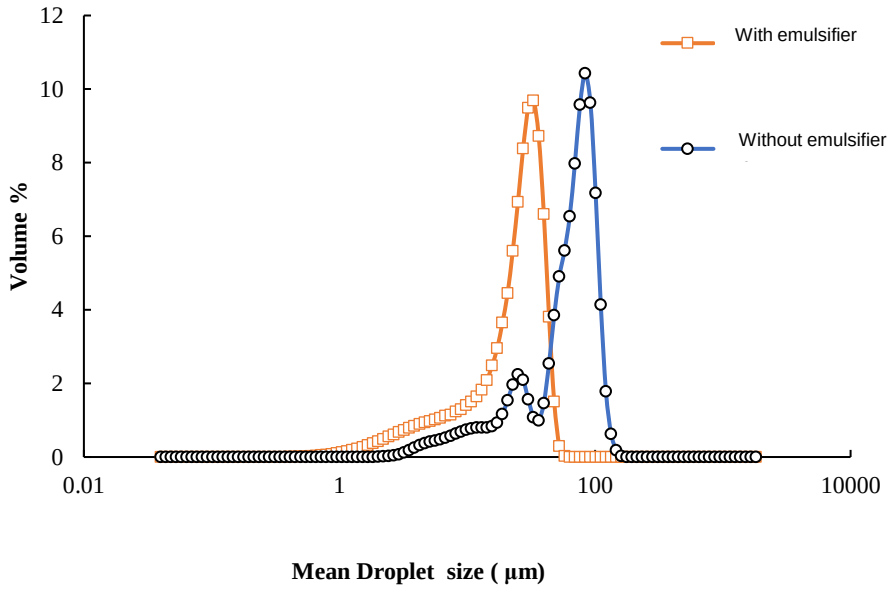


Figure 2.16: Droplet size distribution of O/W emulsion

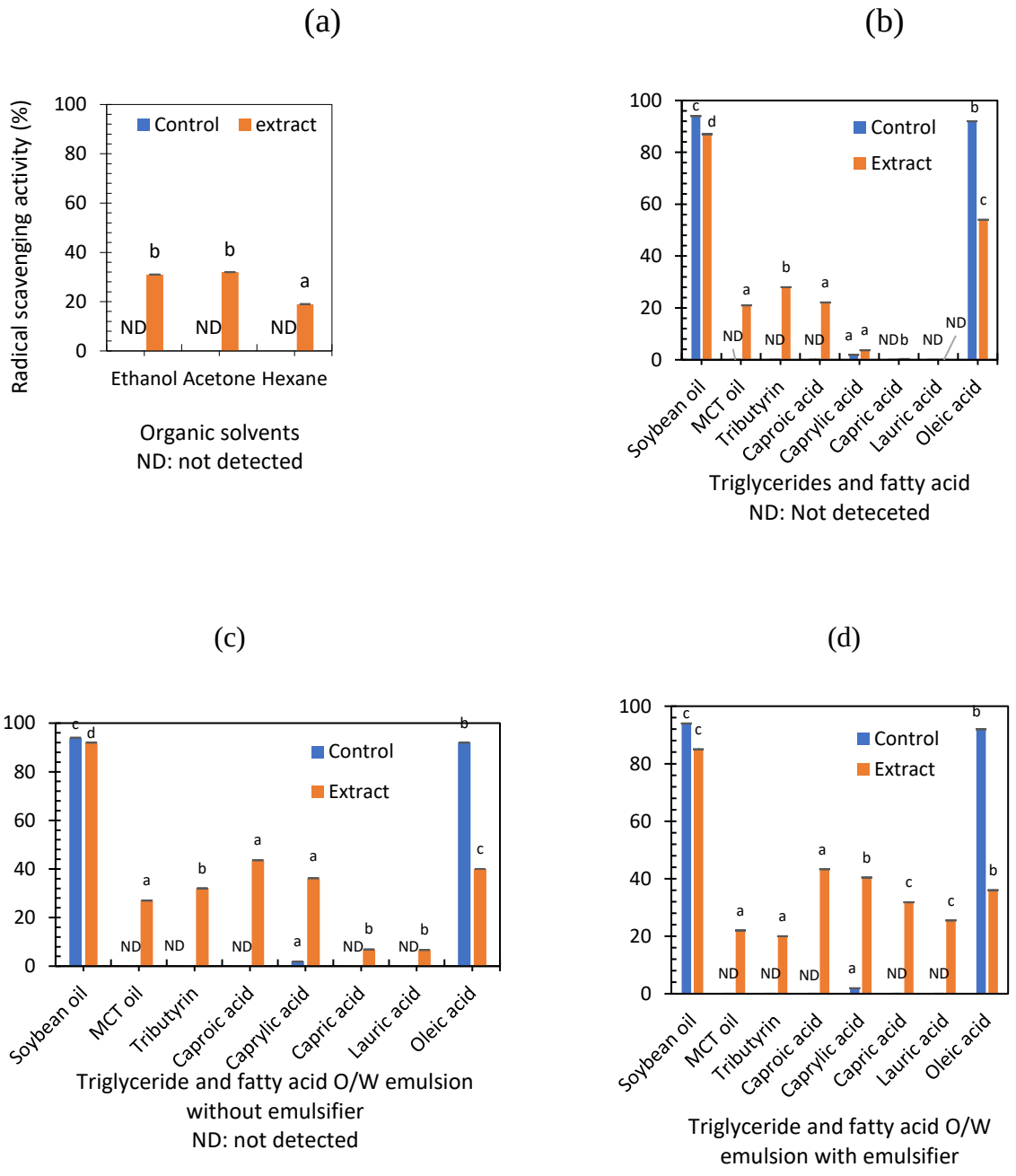


Figure 2.17: Extraction of antioxidant of tomato processing residue using (a) organic solvents, (b) triglycerides and fatty acids, (c) triglycerides and fatty acids-based O/W emulsion without emulsifier and (d) triglycerides and fatty acids-based O/W emulsion with emulsifier extraction methods

Chapter 3

Effect of different extraction conditions of lycopene recovery and antioxidant activity.

3.1 Introduction

Tomato (*Solanum lycopersicon*) is one of the most important crops worldwide, which is rich in natural antioxidant compounds (Brandt et al., 2006b). It is assumed as a functional food that has epidemiological evidence for reducing the risk of certain types of cancers (Cantuti-Castelvetri et al., 2000; Yamaguchi and Uchiyama, 2003). Epidemiological and nutritional studies proposed that within biological systems, the carotenoid in natural tomatoes is the most effective singlet oxygen quencher of all carotenoids (Zhang et al., 1991). In the last decades, abundant research has been performed on the valorization of agro-food products and their industrial by-products. The valorization purpose was the recovery of natural antioxidants and their use as substitutes for synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Importantly, synthetic antioxidants have been recently declared to be liver-damaging and carcinogenic. Therefore, they have been regulated in many places of the world (Dolatabadi et al., 2016).

Lycopene ($C_{40}H_{56}$) is a natural lipophilic antioxidant, which is a symmetrical and acyclic pigment. It is generated in plants as a photosynthesis pigment complex. Lycopene can be found in mature tomato, papaya, pink grapefruit, guava, and watermelon, giving them a distinctive red color (Rao and Agarwal.,1999; Bramley., 2000). It has a significant antioxidant impact as a precursor of β-carotene and is stable in cold, dark environments away from oxygen and light (Urbonaviciene et al., 2012). Atherosclerosis, cardiovascular disease, and some forms of cancer, such as stomach, prostate, and breast cancer, can all be mitigated by lycopene (Jain et al., 1999; Tsushima et al., 1995). It has previously been used as a natural food colorant, but it has recently attracted attention as a pharmaceutical component (Choudhari and Ananthanarayan, 2007).

According to the literature review, various extraction strategies for recovering lycopene have already been developed. However, almost all of the approaches have been proven to be economically unviable for large-scale manufacturing. Lycopene is frequently recovered using toxic organic solvents such ethanol, acetone, n-hexane, and ethyl acetate. Hazardous solvents have several disadvantages, include hazard, solvent remains in the completed project, and environmental contamination. New extraction methods assisted with enzyme, microwave, ultrasound, and emulsifier pretreatments are being studied for carotenoid recovery from tomatoes (Amiri-Rigi et al., 2016; Lianfu and Zelong, 2008). Supercritical fluid extraction is another method for lycopene extraction that enhances lycopene extraction and permits teamwork at moderate conditions. Supercritical fluids are reportedly excessively expensive, which renders the actual system disagreeable and financially unappealing for huge production, according to various estimations. (Naviglio et al., 2008).

In this current research, oil-in-water emulsion-assisted extraction was estimated as a substitute for the organic solvent extraction method. Because of the existence of both lipophilic and hydrophilic domains, this approach is regarded as a green and unique extraction technology for its capacity to incorporate various bioactive (Roohinejad et al., 2014). Emulsion technology is well-known in food science. It possesses exceptional physicochemical properties, including the ability to generate optically clear and colloids that are highly stable, have a high level of absorption for both hydrophilic and lipophilic elements, and improved enzymatic and chemical reaction efficacy (Abbasi and Radi, 2016). Previous studies have reported that The microemulsion technique has been successfully used for the extraction of diverse organic compounds, such as lycopene from tomato peels (Papaioannou and Karabelas, 2012), proteins, enzymes, phenols from liquids (Materna and Szymanowski, 2002), and antioxidants from wine slime (Chatzilazarou et al.,

2010). Due to the limits of current lycopene extraction methods that use dangerous solvents, the industry is calling for a greener, safer, and more effective procedure. Moreover, extraction conditions also have great effects on extraction recovery.

Therefore, in this present study, we intended to extract lycopene from tomato processing residue using different temperatures and times using lauric acid and soybean oil O/W emulsion without an emulsifier.

3.2 Material and methods

3.2.1 Materials

Dried tomato processing residue was provided by Pran Food Ltd, Ekdala, Natore, Bangladesh. Hexane, ethanol (99.5%) acetone, soybean oil, and lauric acid were obtained from Wako Pure Chemical Industries, Ltd. (Osaka Japan). A Milli-Q system (Sartorius, Arium® pro, Goettingen, Germany) generated deionized water (18 MΩ cm) .

3.2.2. Preparation of tomato processing residue powder

Dried tomato processing residue with a moisture content of approximately 7.6 % wt was grounded (Ultra Centrifugal Mill ZM 200, Retsch GmbH & Co. KG) into a homogeneous powder with a particle size of 500 µm and kept at -20 °C for further evaluation.

3.2.3. Total lycopene quantification

With minor adjustments from Fish et al. (2002), the overall lycopene concentration in the tomato processing residue was examined. In a summary, 3 mL of Milli-Q water was added to the solution and stirred for an additional 5 minutes after 2 g of tomato processing residue powder was extracted with a 20 mL fresh combination of acetone, hexane and ethanol, (1:2:1) under continuous stirring (700 rpm) for 15 min at 25 °C. The mixture was then let to spontaneously split into polar and nonpolar phases by resting at 25°C for 5 minutes. The upper phase was filtered through a hydrophobic membrane (0.45 m) and the absorbance was assessed at 503 nm using a UV spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The bottom phase was re-extracted until it left a whitish residue. The lycopene content of the tomato processing residue was calculated following Equation (1):

$$Lycopene\ content\ (\mu g/g) = \frac{A_{503} \times 31.2 \times \text{Dilution}}{Amount\ of\ sample} \quad (1)$$

Where A_{503} and 31.2 are the absorbance of the extract at 503 nm and a constant.

3.2.4. O/W emulsion-assisted extraction

As in the previous chapter, O/W emulsion-assisted extraction was carried out. In order to prepare O/W emulsions, 30 mL of the fatty acid or triglycerides phase (soybean oil or lauric acid) was homogenized with 70 mL of the aqueous phase, either with or without 0.1% (w/w) saponin emulsifier. The emulsions were generated in a rotor-stator mixer for 5 minutes at 7000 rpm (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland). Then, created emulsions (100 mL) and 2 g of powdered tomato processing residue were applied, and the mixture was agitated for 3 h at 50, 60, 70, or 80 °C at 750 rpm. The supernatants were examined to determine their lycopene concentration after the solid particles had been removed.

3.2.5 Lycopene recovery

The lycopene recovery $R(\%)$ from tomatoes was revealed utilizing Equation (2):

$$R\ (\%) = C/C_t \times 100 \quad (2)$$

where C is the lycopene amount in tomato processing residue (µg/g), C_t is the initial lycopene amount in tomato processing residue (µg/g).

3.2.6 Antioxidant activity

According to DPPH free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl) (Górnaś et al., 2014; Shen et al., 2010), the antioxidant activity assessment was accomplished with slight adjustments. In a 3 mL ethyl acetate solution (0.1 mM in ethyl acetate), 1 mL extracts of the given solvent extract were added. After that, the mixture was placed in a dark room for 30 minutes. 1 mL ethyl acetate in 3 mL DPPH radical solution was used to make a blank solution. In order to quantify the absorbance, a UV spectrophotometer was used at 517 nm (V-530, Jasco Corporation,

Tokyo, Japan). Each sample was measured three times. The following equation was used to represent the results in terms of radical scavenging activity:

$$\text{Radical scavenging activity} = A_{control}\text{-}A_{sample}/A_{control}\times 100 \quad (3)$$

3.2.7 Statistical analysis

Each experiment is carried out in at least three duplicates, and the mean and standard deviation of the findings are presented in this research. Using Statistic 8.1 software, the analysis of variance (ANOVA) was used to compare the lycopene content in various conditions at a 95 % confidence level ($p < 0.05$). (Tallahassee, USA).

3.3. Results and discussion

3.3.1. Effects of temperature and time on lycopene recovery and antioxidant activity

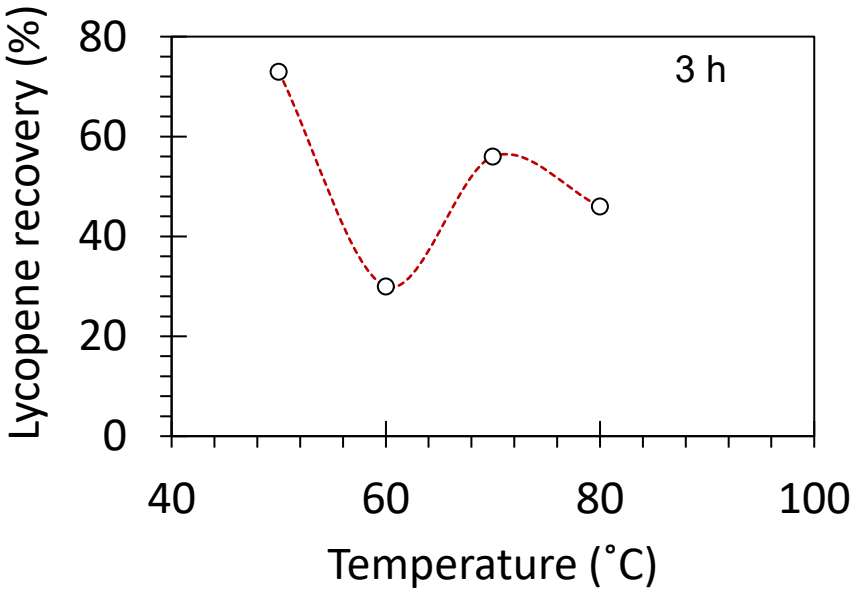
Figure 3.1: a show the effects of extraction temperature and time on lycopene recovery from tomato processing residue using lauric acid-based oil-in-water emulsion as a model emulsion-based extraction system. As shown in Figure 3.1: a, the lycopene recovery decreased while the temperature was increased. The highest lycopene recovery was observed at 50 ˚C. This investigation agrees with previous research, which mentioned that a temperature greater than 65 ˚C resulted in carotenoid degeneration. However, the antioxidant capacity of soybean extract solution increased by 95%. at 60 ˚C (Figure 3.2: a)

On the other hand, the effects of extraction time were investigated at 50 ˚C for lycopene recovery. As shown in figure 3.1:b, the lycopene recovery was enhanced at 2 h extraction. The lycopene recovery was decreased after increasing the extraction time and suggesting a chemical degradation of the extracted compound. As for antioxidant activity (Figure 3.2: b), 3 h was found as the best condition.

3.4 Conclusion

In this study, high lycopene recovery (77%) and antioxidant activity (95%) were found at 50 ˚C for 2 h and antioxidant activity at 60 ˚C for 3 h. Therefore, the extract obtained using lauric acid and soybean oil O/W emulsion was used to assess the stability over time of lycopene and antioxidant activity and the following experiment was carried out under these conditions.

(a)



(b)

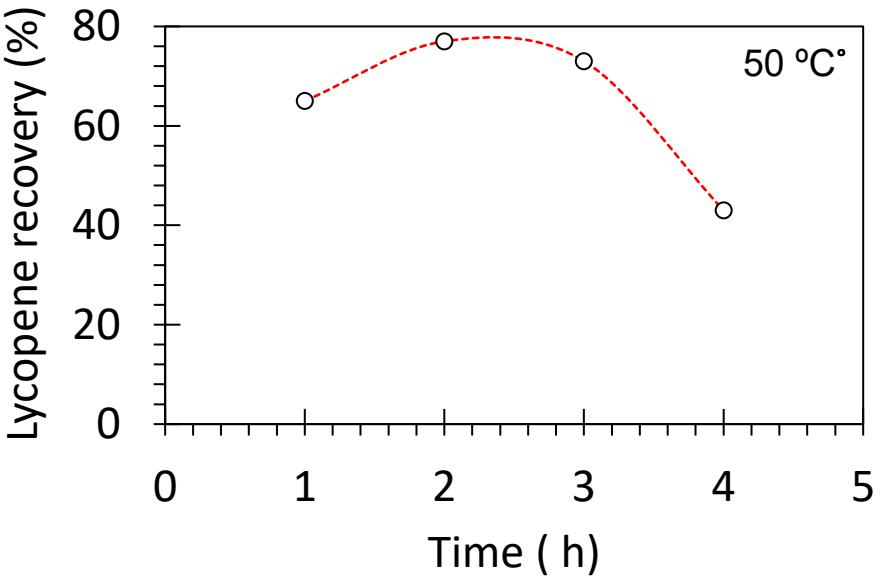
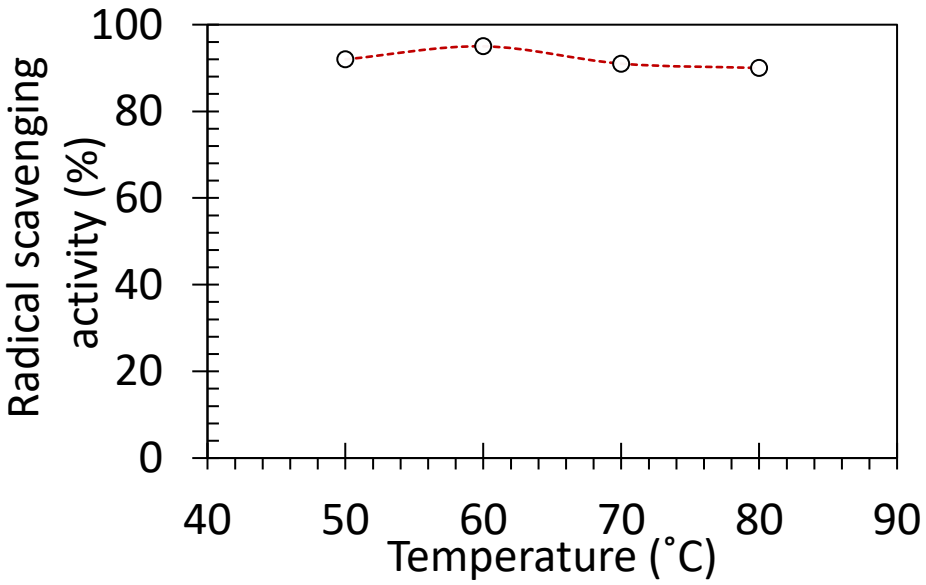


Figure 3.1: Extraction of lycopene from tomato processing residue using different (a) temperatures and (b) time.

(a)



(b)

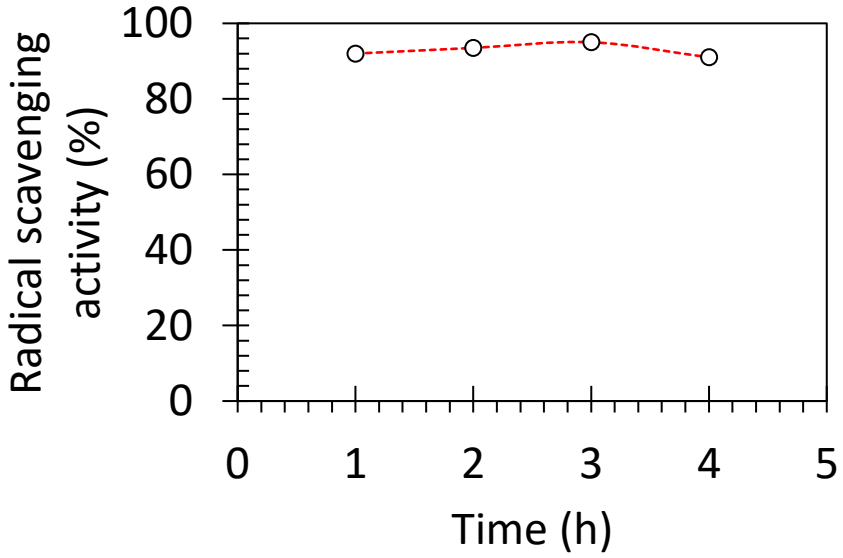


Figure 3.2: Antioxidant activity of soybean oil-based O/W emulsion extract of tomato processing residue using different (a) temperatures and (b) time.

Chapter 4

Chemical stability of lycopene and antioxidant activity of the extract

4.1 Introduction

Lycopene ($C_{40}H_{56}$) is a natural carotenoid that gives tomatoes their rich red color. (Omoni & Aluko, 2005). In epidemiological and clinical investigations, lycopene appears to protect against cardiovascular disease (Cheng et al., 2017; Senkus et al., 2018) cancer (Rowles III et al., 2017; Trejo-Solís et al., 2013; Wang et al., 2015), and neurodegenerative disorders such as Huntington's, Parkinson's, and Alzheimer's diseases (Chen et al., 2019; Sang Cho et al., 2018). Lycopene is powerful antioxidative strain, which is frequently connected to the improvement of the previously mentioned diseases. Furthermore, current research has indicated that lycopene aids in the induction of apoptosis, the inhibition of cell growth, and the expansion of intercellular gap-junctional communication. (Hantz et al., 2005; Zefferino et al., n.d.).

With 13 carbon-carbon double bonds, 11 of which are conjugated, lycopene (also known as, ψ,ψ-carotene) is a tetra-terpenic acyclic hydrocarbon. Lycopene is one of most effective antioxidants because of its high amount of recombination, which also gives it a good antioxidant impact. Singlet oxygen ($^1O_2$) is a reactive chemical that can harm lipids, proteins, and nucleic acids. Lycopene is very efficient at quench molecular oxygen ($^1O_2$)(Przybylska, 2020). The in vitro investigations have evaluated the lycopene's ability to quench singlet oxygen as twice as high as that of β-carotene and 10 times higher than that of α-tocopherol (Di Mascio et al., 1989). It can also help defend LDL cholesterol from free radical damage (Cheng et al., 2017), by preventing oxidation and deposition in the arterial wall, which leads to the formation of atherosclerotic plaques (Cervantes Gracia et al., 2017). The aforementioned findings have raised demand for lycopene-rich medicines, functional ingredients, and beauty products. As a result, the quantity and profitability of the lycopene market have increased quickly. (Ciriminna et al., 2016). Therefore,

the establishment of a novel technology for lycopene recovery seems vital for solubilization and bioavailability.

The majority of natural lycopene is extracted from tomatoes and tomato residue(Zuorro et al., 2011). The tomato, which is frequently recognized as a functional ingredient with a variety of biological features, is one of the crops with the highest concentration of natural antioxidants, such as reducing the risk of certain types of cancer (Brandt et al., 2006a). Tomatoes and tomato-based products contribute to more than 85% of human dietary lycopene consumption, which accounts for about 80%–90% of all tomato carotenoids (A. V. Rao & Agarwal, 2000).

According to the literature review, various extraction strategies for recovering lycopene have already been developed. However, almost all of the approaches have been proven to be economically unviable for large-scale manufacturing. Lycopene is frequently extracted using toxic organic solvents such ethanol, acetone, n-hexane, and ethyl acetate. Hazardous solvents have a number of drawbacks, such as poisoning, chemical residue in the final product, and removal complications. On the other hand, other extraction methods assisted with enzyme, microwave, ultrasound, and emulsifier pretreatments have been studied for carotenoid recovery from tomatoes (Amiri-Rigi et al., 2016). Supercritical fluid extraction is another method for lycopene extraction that enhances lycopene extraction and permits teamwork at moderate conditions. Supercritical fluids are reportedly excessively expensive, which renders the actual system disagreeable and financially unappealing for huge production, according to various estimations.

Microemulsions have been used in recent years to extract a variety of bioactive substances. For instance, using an emulsifier in microemulsion technique, canola oil was recovered from canola plant seeds. (Roohinejad, 2014). While utilizing non-ionic emulsifier, lycopene and β-

carotene were recovered from carrot pomace and tomato peels, correspondingly. (Papaioannou & Karabelas, 2012; Radi & Abbasi, 2018).

In general, the emulsion is known as a heterogeneous system in which with an emulsifier, at least one insoluble liquid is disseminated into the other in the form of drops. This procedure is regarded as a green extraction approach. Due to the existence of both hydrophilic and lipophilic domains, it may incorporate a variety of bioactive chemicals (Roohinejad, 2014). The oil-in-water (O/W) emulsion also contains a significant quantity of protic solvent water, which has the ability to give and accept hydrogen bonds. This property may allow tomato fruits' cell walls to expand, enhancing the recovery of lycopene. Three solvent characteristics that largely affect the process of cellular expansion are basicity, molar mass, and hydrogen bonding tendency. (Flanagan et al., 2006; Papaioannou & Karabelas, 2012). The enlargement process is further influenced by the protic or aprotic character of the liquid.

Although raising the osmotic pressure and resulting in cell elongation by the use of synthesized emulsifiers in microemulsion-based extraction helps to increase total extraction recoveries (Radi & Abbasi, 2018). Considering their utilization in high doses in both dietary and skin care applications, there are an increasing number of safety measures. (Fidale et al., 2008). In contrast, there are no reports of lycopene O/W emulsion-based extraction when a synthetic emulsifier is not present. This innovative extraction technique may contribute to lowering the amount of synthetic and dangerous ingredients used in food, medicine, and cosmetic products while also improving their safety and increasing consumer acceptability. One environmentally friendly solvent renowned for its effectiveness in removing bioactive chemicals is lauric acid. Lycopene has a strong affinity for it. Lauric acid is also a dietary supplement of fatty acids with potential nutritional benefits. It is important to note that no prior research has been done to

determine the effectiveness of lycopene extraction using oil-in-water emulsions based on lauric acid or the chemical stability of lycopene extract.

In this chapter, we investigate the chemical stability of lycopene and the antioxidant activity of lauric acid and soybean oil extract, respectively.

4.2 Materials and methods

4.2.1 Materials

Dried tomato processing residue was provided by Pran Food Ltd, Ekdala, Natore, Bangladesh. Hexane, ethanol (99.5%), acetone, soybean oil, and lauric acid were obtained from Wako Pure Chemical Industries, Ltd. (Osaka Japan). A Milli-Q system (Sartorius, Arium® pro, Goettingen, Germany) generated deionized water (18 MΩ cm).

4.2.2 Preparation of tomato processing residue powder

Dried tomato processing residue with a moisture content of approximately 7.04% wt was ground using (Ultra Centrifugal Mill ZM 200, Retsch GmbH & Co. KG) into a homogeneous powder with a particle size of 500 µm and stored at -20 ˚C until further analysis.

4.2.3 O/W emulsion-assisted extraction

As in the prior chapter, O/W emulsion-assisted extraction was carried out. In a summary, O/W emulsions were produced by homogenizing 30 mL of soybean oil or lauric acid with 70 mL of the aqueous phase without the use of an emulsifier. The emulsions were created in a rotor-stator homogenizer (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland) for 5 minutes at 7000 rpm. Then, created emulsions (100 mL) were added together with 2 g of tomato processing residue powder and agitated at 750 rpm for 2 hours at 50°C for lauric acid, and for 3 h at 60°C for soybean oil. The supernatants were subjected to solid particle removal, lycopene content analysis, and kept for 10 days stability at 5 and 25˚C.

4.2.4 Lycopene quantification

With minor adjustments from Fish et al. (2002), the overall lycopene concentration in the tomato processing residue was examined. In a summary, 3 mL of Milli-Q water was added to the solution and stirred for an additional 5 minutes after 2 g of tomato processing residue powder was

extracted with a 20 mL fresh combination of acetone, hexane and ethanol, (1:2:1) under continuous stirring (700 rpm) for 15 min at 25 °C. The mixture was then let to spontaneously split into polar and nonpolar phases by resting at 25°C for 5 minutes. The upper phase was filtered through a hydrophobic membrane (0.45 m) and the absorbance was assessed at 503 nm using a UV spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The bottom phase was re-extracted until it left a whitish residue. The lycopene content of the tomato processing residue was calculated following Equation (1):

$$Lycopene\ content\ \left(\frac{\mu g}{g}\right) = \frac{A_{503} \times 31.2 \times Dilution}{Amount\ of\ sample} \quad (1)$$

Where A_{503} and 31.2 are the absorbance of the extract at 503 nm and a constant.

4.2.5 Lycopene recovery

The lycopene recovery $R(\%)$ from tomato processing residue was defined using Equation (2):

$$R(\%) = C/C_t \times 100 \quad (2)$$

where C is the lycopene amount in tomato processing residue (µg/g), C_t is the initial lycopene amount in tomato processing residue (µg/g) (Amiri-Rigi and Abbasi 2019). In this study, 588.06 µg/g of total lycopene content in tomato processing residue was investigated

4.2.6 Antioxidant activity

According to DPPH free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl) (Górnaś et al., 2014; Shen et al., 2010), the antioxidant activity assessment was accomplished with slight adjustments. In a 3 mL ethyl acetate solution (0.1 mM in ethyl acetate), 1 mL extracts of the given solvent extract were added. After that, the mixture was placed in a dark room for 30 minutes. 1 mL ethyl acetate in 3 mL DPPH radical solution was used to make a blank solution. In order to

quantify the absorbance, a UV spectrophotometer was used at 517 nm (V-530, Jasco Corporation, Tokyo, Japan). Each sample was measured three times. The following equation was used to represent the results in terms of radical scavenging activity:

$$\text{Radical scavenging activity} = A_{control}\text{-}A_{sample}/A_{control}\times 100 \quad (3)$$

4.2.7 Statistical analysis

Each experiment is carried out in at least three duplicates, and the mean and standard deviation of the findings are presented in this research. Using Statistic 8.1 software, the analysis of variance (ANOVA) was used to compare the lycopene content in various conditions at a 95 % confidence level ($p < 0.05$). (Tallahassee, USA).

4.3 Result and discussion

4.3.1 Chemical stability of lycopene and antioxidant activity

As shown in Figure 4.1, lycopene in the lauric acid extract was stable at 5 ˚C compared to 25 ˚C. However, lycopene in lauric acid extract dramatically decreased at 25 ˚C. A previous study also investigated that lycopene at 25 °C degrades mainly through the oxidation process (Hackett et al., 2004).

According to Galicia et al. (2008), enhanced temperature caused an increase in the constant rate of lycopene degradation. Also, according to research by Spagna et al., 2005, tomato polyphenol oxidase causes color changes such as darkening and lycopene degradation. It is therefore strongly advised to inactivate the polyphenol oxidase to protect the stability of the lycopene.

On the other hand, in figure 4.2 antioxidant activity of soybean oil extract was stable during 6 days of storage. In addition, soybean oil has considerable amounts of natural antioxidants, which may enhance the recovery of antioxidant and helps to keep stable during storage (Massana et al., 1991;Mancini et al., 1993; Antoun et el., 1997: Grati-Kamoun et al., 2007). The low dissociation energy of the double bonds in polyunsaturated fatty acids like linoleic and linolenic acids, which are plentiful in soybean oil, are the main contributors to its oxidative instability. On the other hand, they drastically started to diminish after 6 days of storage.

4.4 Conclusion

In this study, lycopene in lauric acid O/W emulsion extract was stable at 5 ˚C compared to 25 ˚C. However, lycopene in lauric acid extract dramatically decreased at 25 ˚C by the oxidation process. Antioxidant activity of soybean oil extract was stable during 6 days of storage and after 6 days of storage, they dramatically started to decrease.

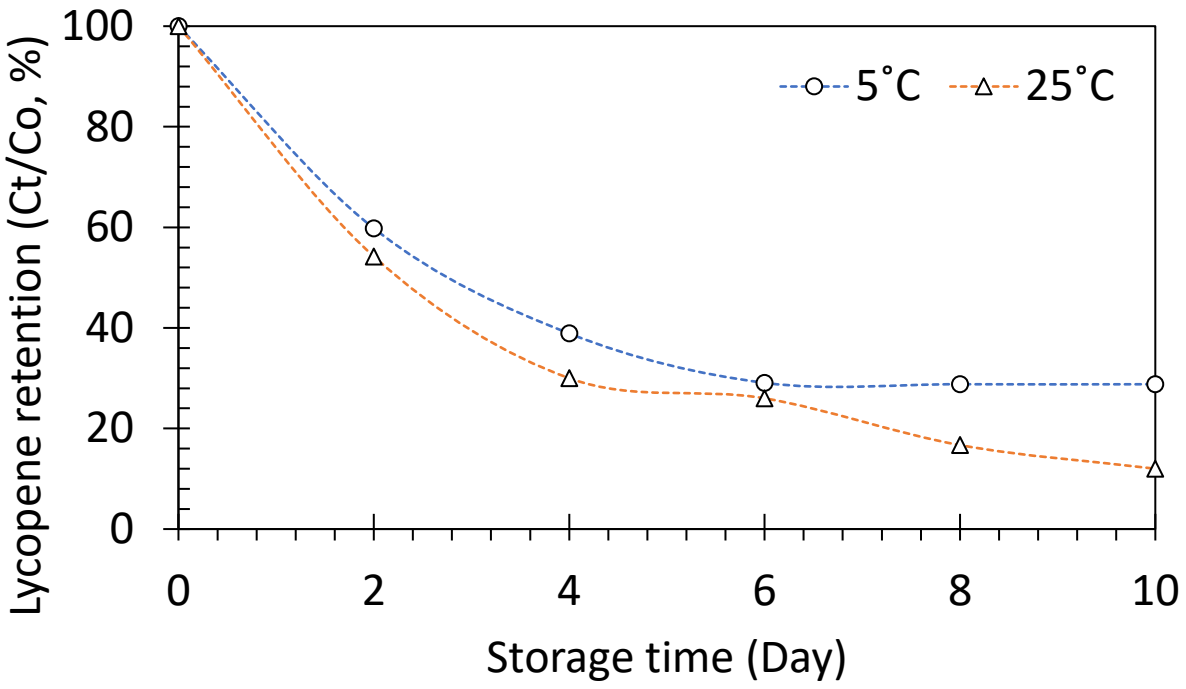


Figure 4.1: Chemical stability of lycopene in lauric acid O/W emulsion extract during storage.

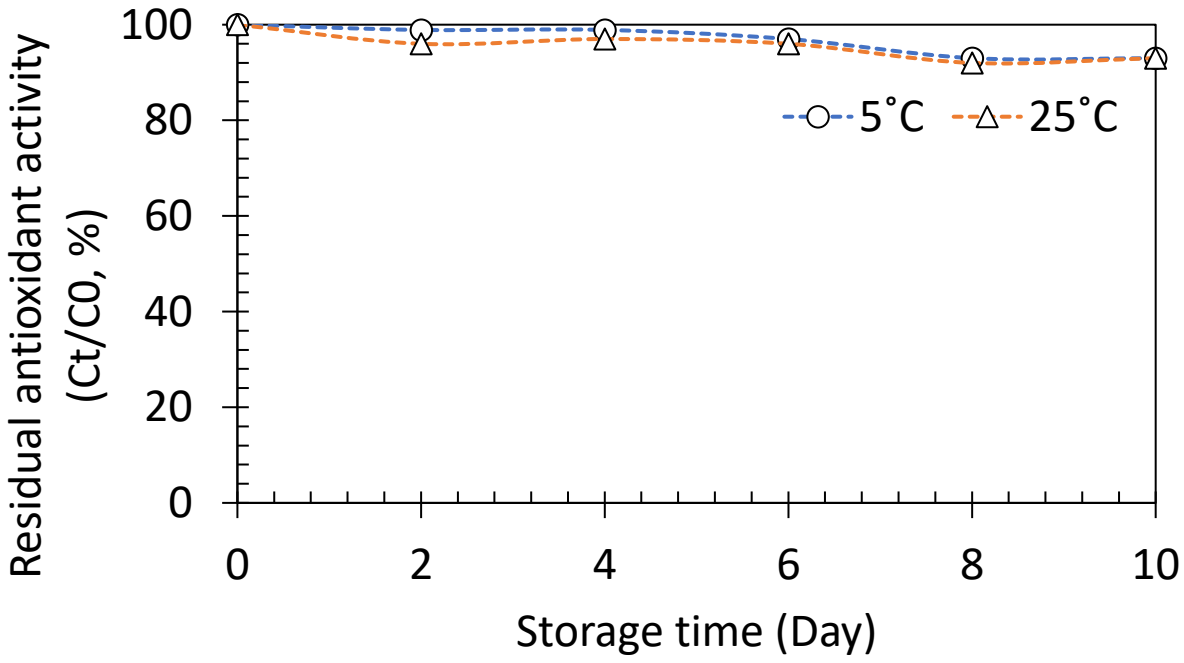


Figure 4.2: Chemical stability of residual antioxidant activity in soybean oil O/W emulsion extract during storage.

Chapter 5

General conclusion and future prospects

5.1 General conclusion

In this study, lycopene was extracted from tomato processing residue using organic solvents, triglycerides, fatty acids O/W emulsion-based extraction. In comparison to organic solvents, triglycerides, or fatty acids, O/W emulsion was typically more efficacious at recovering lycopene. O/W emulsion without an emulsifier enhanced lycopene recovery almost two times more than organic solvents and crude triglycerides extraction, respectively. Enhanced recovery is a result of the O/W emulsion's capacity to expand the cell without the addition of an emulsifier. Cellulose is structured as microfibrils in cellulosic material, with both amorphous and crystalline areas. Slightly thicker fibers are created from microfibrils, which are subsequently merge by hemicelluloses and encased in a cellulosic substrate that simulates a jelly. The quantity of cellulose fiber and the spatial structure of the lignocellulosic materials matrix are regulated by intermolecular and intramolecular interactions. These connections are produced by the hydroxyl groups in the cellulose-1, 4-linked D-glucopyranose units. Plant matrix-penetrating solvent molecules can bind to such hydroxyl groups by adsorption, due to their small size and strong polarity. Adsorption causes some connections to break down, which widens the gap between cellulose fibers and causes the substance to expand. The amorphous portions of cellulose, which are more responsive and susceptible to chemicals, are the only places where expansion may occur. The expansion process is also influenced by the solvent's protic or aprotic character. because complexation solvents like water are capable of both hydrogen bond creation and destruction. As hypothesized in the introduction section, the existence of a large volume of water, swelling behavior during O/W emulsion extraction is greatly amplified and emulsion enhances the surface area by small droplets size. For the lauric acid-based emulsion without an emulsifier, the addition of protic solvent water is thought to allow the tomato processing residue tissue to enlarge. This

expansion helps lauric acid permeate the plant matrix, increasing the effectiveness of lycopene extraction. Furthermore, it was shown that lycopene could not partition to triglycerides or fatty acids if an emulsifier is added. Lauric acid was discovered to be the ideal solvent for lycopene recovery since it is non-toxic and safe for the environment. Therefore, lycopene recovery (77 %) using lauric acid-based O/W emulsion extraction without an emulsifier might be an efficient and promising method that can be used to food supplements and dietary nutritious complements. On the other hand, when compared to other triglycerides and fatty acids, O/W emulsion extraction techniques have the strongest antioxidant activity. In consideration of this, O/W emulsion without emulsifier can also be a green extraction technique for antioxidant activity.

5.2 Future prospects:

The obtained findings of this thesis could provide insight and helpful information for the recovery of natural compounds, since the industry expects a cleaner, safer, and more efficient method. Potential applications can be created according to these findings. Such as cosmetics and pharmaceuticals application. On the other hand, fatty acid-based O/W emulsion extraction without an emulsifier has the potential to be a potentially environmentally friendly method for lycopene recovery and antioxidant activity, which may subsequently be applied in nutritional nutraceuticals and dietary supplements. The extracted lycopene can be used as a modified packaging as well. The molecular behavior of O/W emulsion with or without emulsifier and their influence on the lycopene extraction process, nevertheless, should be the subject of additional investigation.

On the other hand, not only lycopene but also tomato processing residue can use for long-term approaches to make valuable products. Investigation and manufacturing concentrating on developing unique and goods with a high level of value, particularly naturopathic remedies and medicines, are also part of upcoming approaches and initiatives for the use of tomato processing residue. Furthermore, compost made from tomato processing residue is seen as a viable organic waste management alternative because of its availability and capacity to enhance soil properties and vegetation development. Another possibility is the bioconversion of tomato processing residue into sustainable gaseous or liquid renewables, such methane and bioenergy. Bioenergy is a potential, inexhaustible, and long-term energy source that can meet growing energy demands while also addressing the depletion of natural gas Hydrolases enzymes are created in solid-state fermentation from tomato processing residue (Lu et al., 2019). Additionally, tomato processing residue can be used also in food to enhance the dietary fiber antioxidants with attractive color. A developing country such as Bangladesh also produces a big amount of tomato processing residue.

If this residue may use to make some food or add to foods, which may help to get more nutrients as well.

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List of publication

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