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PRODUCTION OF NATURAL FOOD-DERIVED VITAMIN C FROM ORANGE JUICE

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Abstract— Vitamin C is also called ascorbic acid and ascorbate. Usually, vitamin C can be found in citrus fruits, bell peppers, guavas, papayas, cruciferous vegetables, and tomatoes. Synthetic vitamin C supplement is also widely sold in pharmacies. Vitamin C is needed for normal growth and development of the human body, and it also functions as an antioxidant to provide plants protection from side-effects of light during photosynthesis in plants. Shortage of vitamin C in humans may result in scurvy disease. Synthetic and food-derived vitamin C are chemically identical, however there is increasing consumer interest in natural food-derived vitamin C. This chapter provides a review of the technology used to extract vitamin C from orange juice, treatment of extracted vitamin C, quality control tests on the food-derived vitamin C, as well as the advantages of using these technologies to produce food-derived vitamin C.

Keywords— Vitamin C, citrus, orange juice, high pressure (HP) treatment, antioxidant

7.1 INTRODUCTION

Vitamin C is a significant water-soluble nutrient sourced mainly from fruits and vegetables. Plant tissues and most animals can produce vitamin C. Humans and a few vertebrate species could not synthesize this compound in the liver. Vitamin C is important in both plants and animals. In plants, vitamin C plays a role in cell division control, and an antioxidant for stress factors such as ozone and UV radiation [1]. In humans, vitamin C helps the body produce collagen, increase iron absorption, and its antioxidant activity assists in removing reactive oxidative species (ROS), which boosts the immune system and enhances wound healing. Antioxidants are molecules that can avoid or slow oxidative destruction from ROS to lipids, proteins, and nucleic acids. Vitamin C is a dietary antioxidant that is used as a co-factor for several enzymes. The reduced form of vitamin C is the L-ascorbic acid while the oxidized form is dehydroascorbic acid. The l-isomer is the primary active source of vitamin C and is found in the reduced form in human blood [2]. Consumers, public health agencies and the scientific profession have demonstrated growing interest in promoting health and well-being by dietary means. This is largely due to the elevated rates of detrimental dietary-related health disorders such as obesity, late-onset diabetes and cardiovascular disease and their resulting social and economic effects. At the same time, our understanding of the function that fruit and vegetable intake play in sustaining human health and reducing the risk of disease, is increasing. Many epidemiological trials have found that people with higher vitamin C consumption are at reduced risk for many chronic conditions, such as cardiac failure, asthma, cancer, or neurodegenerative disorders [3]. The quality and nutritional status of fresh produce however, is influenced by post-harvest handling and storage conditions. These involve the difference in nutrient content, lack of volatile scent composition and texture products [4].

Orange juice (*Citrus sinensis*) is commonly consumed fruit juice in most part of the world and is especially valued for their flavour, high vitamin C content and natural antioxidants such as flavonoids and phenylpropanoids (Figure 1). A study to compare vitamin C content in citrus fruits (orange, grapefruit, lemon, lime, kaffir lime and musk lime) using titration and HPLC methods showed that orange has the highest vitamin C content (58.30 mg/100g) out of all the citrus fruits tested [5].

As an example, blood orange juice is an Italian product with higher amounts of ascorbic acid and hydroxycinnamic acids relative to blond orange juices (refer to Table 1) [6,7]. The presence of anthocyanins gives the vivid red colour. In the European market, there are three main types of these products: (1) freshly squeezed juices with milk pasteurization, (2) not from concentrate juices (NFCs) gained by freezing after squeezing, and (3) juices reconstituted from concentrate (RFCs). A major part of the market demand is focused on the latter products, because the concentration phase (up to 60 ˚Bx end concentration of dissolved solids) decreases storage volumes (so lowering shipping and storage charge) and promotes preservation. However, a significant loss of volatile organic flavour / aroma elements, along with a minor deterioration of ascorbic acid and natural antioxidants, followed by a certain discoloration and a consequent qualitative decrease, occurs when the processing is performed by conventional multistep vacuum evaporation. Such symptoms are mostly attributed to the conversion of heat to the juice during evaporation [6].

Preserving the fundamental properties of the orange juice is one of the main objectives of production technology; indeed, as most of the orange juice consumed is produced by industrially integrated processes, researchers are continually seeking innovative technological solutions to prolong shelf life without affecting quality. In the quest, the key problems to be addressed during development and storage are inhibition of microbiological spoilage and inactivation of the enzyme. In fact, enzymes such as pectin methyl esterases (PME) are responsible for gelling phenomenon during storage; however, by inducing pectin hydrolysis, they destabilize the cloud fraction and induce juice clarity [7].

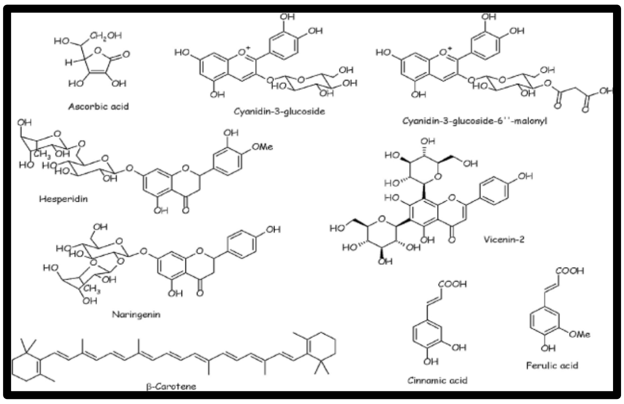

Figure 1: Antioxidant component of orange juices [7]

Table 1: Relative amount of the different Orange Juice's Bioactive Antioxidant (mg/l) [7]

Component	Blond Orange Juice	Blood Orange Juice
Ascorbic acid	150-350	450-650
Hydroxycinnamates	60-70	70-80
Flavanones	55-150	80-110
Carotenoids	4-9	3-8
Anthocyanins	-	50-100

In addition, polyphenol oxides (PPOs) and peroxidases (PODs) cause oxidation reactions of ascorbic acids, polyphenols and carotenoids, resulting in browning and colour changes. The most successful and common means of inhibiting such processes are thermal stabilization treatments, but many undesirable degradation reactions affecting both organoleptic and nutritional aspects are frequently encountered.

The aim of this review is therefore to reflect on the impact of refrigerated storage on vitamin C and antioxidant production of orange juice processed by modern technologies (HP and PEF) relative to orange juice processed by conventional thermal technology as low pasteurization (LPT) [7,8].

7.2 EXTRACTION AND TREATMENT OF CITRUS JUICE

Traditionally, heat treatment for the thermal pasteurization process of citrus juice is performed to prolong its shelf life. High pressure (HP) and pulse electrical field (PEF) are currently being widely researched as non-thermal food storage steps. Several researchers discovered that citrus juice processed by High Pressure and Pulse Electrical Field treatment retains the bioavailability of vitamin C and the antioxidant component of fresh juices in individuals. In addition, there is also study being done on the effect of refrigerated storage on the wellbeing-related properties of freshly pressed orange juice handled by these new technologies. The impact of refrigerated storage on vitamin C and antioxidant production of citrus juice processed by modern technologies (HP and PEF) relative to orange juice processed by conventional thermal technology as low pasteurization (LPT) is assessed [8]. The orange juice production process is characterized by several consecutive steps as shown in Figure 2 [7].

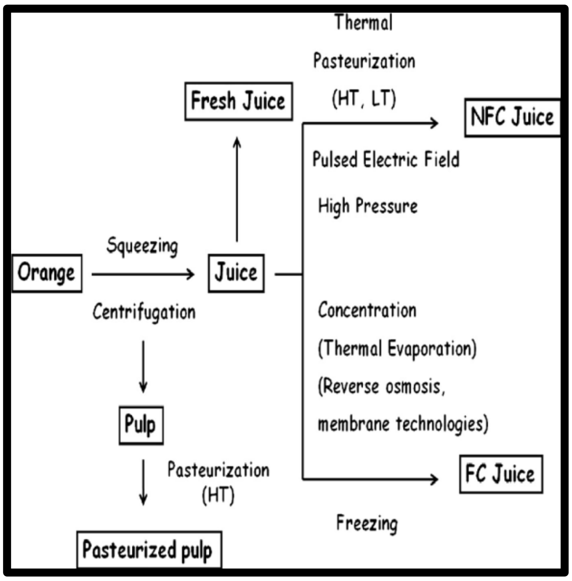

Figure 2: General schematic production process of orange juice [7]

7.2.1 Reagents

Reagents used for the research includes dl-dithiothreitol and DPPH• (2,2-diphenyl-1-picrylhydrazyl) from Sigma (St. Louis, MO); citric acid monohydrate and 0.1 M sodium hydroxide (NaOH) from Pancreac Qumica, Spain; Methanol and sulphuric acid (H2SO4) from Labscan Ltd. (Dublin, Ireland); Acetic acid from Hopkin and Williams (Essex, U.K.); L-(+). Ascorbic acid and meta-phosphoric acid from Merck KGaA (Darmstadt, Germany) [8].

7.2.2 Orange Juice Samples

Orange fruits (*Citrus sinensis* L. Valencia late variety) (Valencia, Spain) were used as samples. Citrus juice was produced by a standard presser usage (Lomi Model 4, Madrid, Spain). After that, it was sieved through 2 millimetres iron sieves. The original characteristics of freshly pressed citrus juice was as shown in Table 2 [7]. Then, high-pressure treatment and PEF treatment were performed for the juice samples.

Table 2: The characteristic in pressed citrus juice [8]

Characteristic	Value[a]
Titratable acidity (g citric acid/100 g of fw)	0.79 ± 0.01
pH	3.51 ± 0.03
Soluble solids (°Brix at 20 °C)	13.31 ± 0.05
Total solids (g/100 g of fw)	13.70 ± 0.25
CIE*Lab* color parameters	
L^*	44.63 ± 0.20
a^*	5.36 ± 0.11
b^*	30.37 ± 0.13
h ($\tan^{-1}(b^*/a^*)$)	79.98 ± 0.18
C $(a^{*2}+b^{*2})^{1/2}$	30.84 ± 0.14
Viscosity (cP)	52.50 ± 4.23

fw: Fresh weight
[a] Values are the mean of three independent determinations ± standard deviation

7.2.3 High-pressure (HP) treatment

Freshly pressed citrus juice samples (250 ml) were packed in no air condition bags which are flexible Doypack (XL size polyskin, Amcor Flexibles Hispania, Spain). After that, the sample was pressurized in a pressure-filled unit (water). This treatment at 400 MPa was conducted in a hydrostatic pressure tank with a volume of 2,350 mL, a nominal pressure of five hundred MPa and a theoretical surface temperature of 95 ˚C. The pressure compartment needs to be heated / chilled to the required degree by means of a thermostat jacket attached to the warm water before pressing. The compression and decompression rate were 2.5 MPa per unit second. Samples were performed at 36 ˚C and controlled at 400 MPa for 1 min. Due to adiabatic strain, the tank was at 40˚C and 400 MPa for its mean temperature. Pressure (MPa), time (minutes) and temperature(˚C) were adjusted in a computer system, continuous audit was also reported throughout every step. These processing conditions were chosen according to the group's preliminary findings based on their impact on the stability of vitamin C in citrus juice counting on the enzymatic inactivation and microbial reduction [8]. Figure 3 shows the high-pressure treatment used in this project.

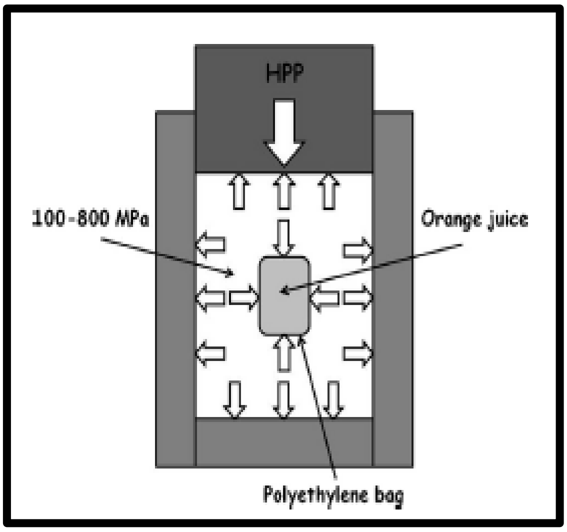

Figure 3: High-pressure treatment [7]

7.2.4 Pulsed electric fields (PEF) treatment

Continuous flow bench scale system was used to conduct this PEF treatment obtained from Ohio State University. There are a series of 8 co-linear treatment chambers in this treatment process. 2 stainless steel electrodes, which are separated in 0.29 cm distance composed in every chamber. The process flow rate was set to 200 mL in a minute and operated using variable speed pump equipment. Between the chambers, the product was cooled directly in the space given which contained an ice-water shaking bath. The condition for the freshly pressed citrus juice to be conducted: 4 µs pulse width, 35 kV/cm electrical field applied in bipolar mode, 750 µs total treatment time and 800 Hertz pulse frequency. The temperature must not exceed 50˚C. This treatment was chosen based on past group findings showing the good enzymatic inactivation and microbial reduction in citrus juice [7]. Figure 4 shows the orange juice that has been treated in PEF treatment.

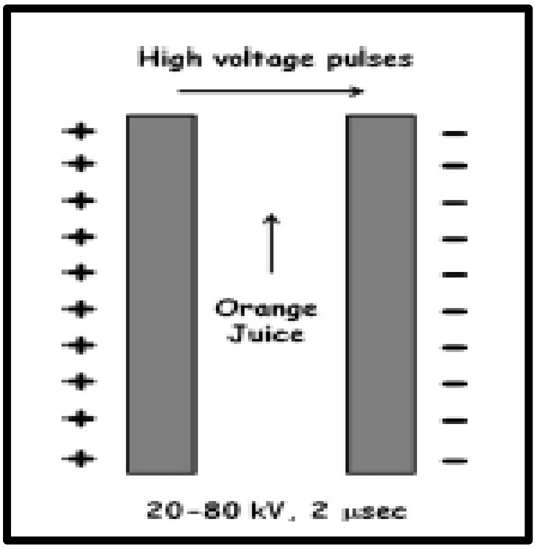

Figure 4: Pulsed electric fields treatment [7]

7.2.5 Low pasteurization (LPT) treatment

The same bags that have been used in HP treatment were packed with freshly pressed citrus juice samples, heating up to 30 seconds with the temperature of 70˚C. Based on the findings in a previous study, LPT treatments have been chosen from the various thermal treatments according to the result of past research [8].

7.2.6 Refrigerated storage

The samples of citrus juice were sterilized, packed, with impermeable tubes under the nitrogen flow and processed in the duration of 40 days with 4˚C temperature right after the treatment. Ascorbic acid, total vitamin C and radical scavenging properties were measured in untreated (freshly pressed) and treated (HP, PEF and LPT) citrus juice. An analysis of storage at 4 ˚C after the treatment (D0) and after 10 (D10), 15 (D15), 20 (D20) and 40 (D40) days was conducted [8].

7.2.7 Color Measurement

Tristimulus reflectance colorimeters were used to test the color of citrus juice calibrated with tile which is white standard (X=82.51; Y=84.53; Z=101.23). The extracted juice was put in Petri dishes and filled to the top, and the color was reported using the standardized color space of CIELab. L* (lightness), a* (green–red tonality) and b* (blue– yellow tonality) values were reported and results were represented as hue angle h=tan−1 (b*/a*) and saturation (or chroma) C=(a*2+b*2)1/2.

7.2.8 Viscosity

Approximately 35 ml of orange juice was tested for its viscosity using viscometer and the unit is centipoise (cP) [8].

7.2.9 Vitamin C determination

HPLC has been used to determine the ascorbic acid and total vitamin C (ascorbic acid + dehydroascorbic acid) concentration. The technique used to assess total vitamin C was to reduce dehydroascorbic acid to ascorbic acid by using dl-dithiothreitol as a reductive agent, as updated in the Sanchez-Mata et al. procedure. An amount of 50 mL of each citrus juice was homogenized with 40 mL of the solution that had been extracted (3% meta-phosphoric acid + 8% acetic acid). Under suction, the resulting combination will pass through to be filtered and adjusted to 100 mL with distilled water. Samples were passed through a membrane filter of 0.45-µm, and duplicates samples of 20 µl were screened and examined using HPLC. The findings will be represented in mg of ascorbic acid/100 mL (Table 3) [8].

An aliquot of the previous solution was taken to respond with 2 ml of 20 mg / milliliters of dl-dithiothreitol for 2 hours at room temperature and darkness. Since this process, dehydroascorbic acid has been converted to ascorbic acid. Samples were purified via a 0.45-µm membrane filter, and duplicates of 20 µl for each sample were examined by HPLC. The findings will be represented in mg of total vitamin C/ 100 mL [8].

Table 3: Vitamin C content of orange juice (mg/100 mL) [8]

Table 2 Vitamin C content (mg/100 ml) of orange juices

Treatments	Storage days (4 °C)	L-Ascorbic acid (mg/100 ml)	Total vitamin C (mg/100 ml)
Freshly squeezed	0	47.01 ± 0.63bcC	47.96 ± 1.05cdB
	10	47.15 ± 1.22cB	48.42 ± 0.62dB
	15	45.91 ± 0.90bcC	46.62 ± 0.87bcC
	20	45.59 ± 0.33bD	45.76 ± 0.51bD
	40	42.65 ± 0.85aC	43.89 ± 0.73aC
LPT (70 °C/30 s)	0	46.33 ± 1.20dBC	47.11 ± 1.23dB
	10	44.77 ± 1.61dB	46.05 ± 1.39dB
	15	42.36 ± 0.40cB	43.76 ± 0.21cB
	20	37.83 ± 0.52bB	40.19 ± 0.53bB
	40	33.44 ± 0.43aB	35.88 ± 0.23aB
HP (400 MPa/40 °C/1 min)	0	44.78 ± 0.68bcAB	46.48 ± 0.76cAB
	10	45.39 ± 0.86cB	47.05 ± 0.54cB
	15	42.90 ± 0.24bcB	44.88 ± 1.82bcBC
	20	42.41 ± 2.07bC	43.66 ± 0.26bC
	40	36.04 ± 2.78aB	37.86 ± 2.14aB
PEF (35 kV cm^{-1}/750 µs)	0	43.39 ± 1.28eA	44.71 ± 1.21eA
	10	41.31 ± 1.74dA	42.44 ± 2.05dA
	15	36.73 ± 0.11cA	38.36 ± 0.23cA
	20	33.50 ± 0.93bA	35.10 ± 0.93bA
	40	29.12 ± 0.69aA	30.26 ± 0.90aA

Values are the mean of three independent determinations ± standard deviation. Different small letters in the same compound and treatment during storage indicate significant differences ($P<0.05$). Different capital letters in the same compound and day indicate significant differences ($P<0.05$). HP: high-pressure; PEF: pulsed electric fields; LPT: low pasteurization

7.2.10 Chromatographic Procedures

For this step, the examination was performed by using quaternary solvent delivery controller pump Model 1050 Hewlett-Packard. A manual injector (Rheodyne) was used to inject the sample to the column with a sample loop of twenty microliter. Adjusted to pH 2.5–2.6, ascorbic acid separation was conducted by HPLC using reversed phase C 18 Hypersil ODS (5 µm) stainless steel column (250×4.6 mL). The solvent system used was an isocratic solution gradient of 0.01 % H2SO4. Setting flow rate at 1 mL per minute. Setting 245 nanometres of UV-vis photodiode array detector; the result of UV-vis spectra and chromatographic result obtained, stored and integrated with the Hewlett-Packard ChemStation and relevant applications. Identification of ascorbic acid was done by HPLC by contrasting the retention period and the UV-vis absorption spectrum with those of the standard previously referred to [8].

7.2.11 Determination of Antioxidant Activity

Determination of free-radical scavenging ability can be evaluated using a stable radical 2,2 -diphenylpicrylhydrazile. In short, the sufficiently diluted citrus juice sample was mixed with 3.9 mL of DPPH• (0.0030 g per 100 mL) in methanol. The absorbance was measured at 515 nm for 15 seconds intervals using an Amersham Pharmacia Biotech spectrophotometer, with 6 cell holders until the process reached the time where steady state occurs. The absorption change will be assessed at 25℃. On the day of preparation, the citrus juice samples that have been diluted earlier were used. The curve of calibration at 515 nm was used with DPPH• to measure the concentration of DPPH• in the medium of the reaction. The percentage of balance DPPH• was calculated as below:

$$\% \text{ DPPH•Rem} = ([\text{DPPH•}]_t/[\text{DPPH•}]_{t=0}) \times 100$$

The percentage of balance DPPH• was evaluated by using a simple regression analysis and the concentration of orange juice. EC50 values, that reflect 50% reduction of starting DPPH• radical, and expressed in mL of juice/gram of DPPH• in the medium of the reaction. The time required to achieve a steady state at a concentration of EC50 (TEC50) was measured graphically (Table 4) [8].

Table 4: Impact of Processing on Orange Juice's Antioxidant Components [8]

Treatment	Vitamin C	Flavanones	Flavones	Carotenoids	Hydroxycinnamic Acids	Anthocyanins	TAA
HPT (95°C, 30s)	Decrease	No change	No change	Decrease	Decrease	Decrease	Decrease
LPT (75°C, 30s)	Slight decrease	No change	No change	Slight decrease	Slight decrease	Decrease	Slight decrease
HPP	No change	Increase	—	Increase	No change	No change	No change or slight decrease
PEF	Slight decrease	No change		Slight decrease	—		No change
Concentration by TASTE	Slight decrease	No change	No change	Slight decrease	Slight decrease	Decrease	Decrease
Concentration by UF-MD	Slight decrease	No change	No change	No change	No change	Slight decrease	No change

TAA, total antioxidant activity; HPT, high pasteurization temperature; LPT, low pasteurization temperature; HPP, high-pressure processing; PEF, pulsed electric fields; TASTE, Thermally Accelerated Short-Time Evaporators; UF-MD, ultrafiltration and and membrane distillation.

7.3 ADVANTAGES

7.3.1 Advantages of HP and PEF as non-thermal processing technology in the production of vitamin C as antioxidant from an orange juice

High-pressure (HP) processing technology is widely used in the production of food as it has a higher performance that can tolerate nutritional value, flavor, texture, scents and thus prolong shelf life [9]. Pulse Electric Field (HEP) processing technology is a non-thermal processing technology requiring a strong electrical field (10 to 100 kV / cm) with a shorter pulse (1–10 μs) through the product [10]. Thus, processing equipment for the High Pressure (HP) and Pulse Electric Field (PEF) provides a good chance to pursue innovative product production with a better nutritional profile and a prolonged lifespan.

HP and PEF show equivalent quality product attributes in most cases. Both HP and PEF are known to be promising for use in the production of vitamin C as antioxidants from orange juice. Since consumers are searching for healthy nutrients, microbiologically efficient nutrient storage technology is well established. The HP and PEF processing range is implemented as part of these modern manufacturing and preservation technologies or in combination with the other methods [9,10]. The advantages compared to other technologies, especially thermal preservation methods, are elaborated below.

(i) Maintain the bioactive compound

Orange juice is one of the major suppliers of bioactive compounds such as carotenoids, antioxidant flavanones, glycosides and other phytochemical compounds [7]. HPP and PEF offers an advantage of minimal heat penetration and heat damage to raw material or selective products as it retains the freshness, flavour, colour and vitamin C content in orange juice [9,10]. This non-thermal processing technology acts as alterations to the thermal conventional treatment that used the high heat mechanisms. One of the limitations recognized in thermal processing is lower product's nutrient value due to the heat damage. Most of the desired content in products such as bioactive compounds are degraded due to the sensitivity of bioactive compounds to the heat.

Bioactive substances are the products that affect the body or other cells or tissues. These bioactive compounds can be correlated with a reduction in the incidence of disease in individuals. For example, some antioxidants act as defence mechanisms that boost oxidative stress response, avoid harm or accelerate cure [2]. To be short, by using the HP and PEF as an alternative to thermal treatment capable of preserving the bioactive compound for various benefits in aspects of human health.

(ii) Reduction in microbiological loads

Microbiological loads are described as the number and type of microorganisms contaminating products. An easier and accurate technology to reduce microbial loads is important in production of vitamin C. In this case, High Pressure and Potential Electric Field processing shows the most efficient technologies to reduce microbiological loads and microbial inactivation. HP processing can transmit the high pressure from 300 up to 700 MPa by using the water as the medium to the selective product [9]. The high pressure charged towards the product resulted in reduction in microbial loads. HP processing is a non thermal process which usually operates at room temperature to inactivate the bacterial cells, yeasts and molds, without the use of heat. The ability of HP processing to reduce the microbial activities without the use of heat mechanism is associated with the fresh quality of the product especially in terms of colour, texture and flavour.

Another non-thermal processing technology that shows the same potential as HP technology in terms of reducing microbial loads is PEF. In a PEF processing, the selected product is treated with two electrodes giving electrical pulses in the chamber. The use of short pulses of electricity is able to constraint microbial activities. PEF superiority induces minimum negative impacts for the quality profile of the product [10]. In short, HP and PEF have been shown in comparison with traditional thermal methods as it kills microorganisms and maintains better original colour, flavour, texture and nutrition. The goal of HPP and PEF will be to offer high quality consumer products, including application to product constituents of highest quality and acceptability.

(iii) Competitive market value

Market value for each product is one of the secrets to business success that can enhance the product profile, capability, and customer acceptability. From the market value of products that suits the approach to the target customer, competitors and understanding the relationship between quality and price value of products seen in this development area able to fulfil the customer demand for healthier, fresher, better and more nutritious customer products. This high-pressure processing (HPP) and potentials of electricity field (PEF) technologies have been revealed by the published research to inactive the pathogenic microorganism at near ambient temperature, can extend shelf life product and fresh like character product in line with the competitive market value.

(iv) Energy Consumption

Energy use is an incredibly significant aspect that should be addressed in the current and future industrial environment. Energy consumption is the amount of energy or power used to perform an action or operate, manufacture the product. Energy consumption in production of vitamin C consists of a combination of various forms of work energy, for example for pasteurization, thermal or drying processes and electricity for illumination, ventilation devices, and cooling systems. Unnecessary electricity consumption may be reduced by implementing power management. The energy consumption of production could be cut with a high percentage through recirculation and heat recovery [11]. Application of modern innovations as non-thermal technologies or low energy pre-treats, i.e. HPP and PEF, will provide strong incentives for the continuous improvement and energy quality on production of vitamin C as antioxidants.

The major energy source used depends significantly on the form of process and technologies employed in production and product. For instance, significant variations in energy production requirements due to concern over energy losses as well as variations in equipment. The most significant operating considerations were the age of the installed equipment, the costs for resources, and the form of heat transmission mechanism and the usage of usable capacity. The broad range of appliances used, and the large variety of applications, services and techniques provides challenges for the energy consumption analysis. Its economic performance in terms of manufacturing prices, the environmental quality and commodity added benefit must be shown when implementing modern technologies to substitute or improve existing methods. In this context, the suitable use of PEF and HP for manufacturing, and the opportunity in saving energy and development sustainability are required to be priorities [11].

PEF applications may need the additional power input in the form of electric power, but it shows many benefits upon the effect of total energy consumption for operation such as mass transfer process, extraction or pressing. For example, PEF used to press apple pieces at a pressure of 3 MPa achieved a higher yield of 12% [12]. This indicates that PEF operates at lower energy for fruit pressing. Another example shows the manufacturing of sugar beet, one of the most energy consuming industries. It was reported that the use of thermal processing became unnecessary after PEF technology with quality preservation and energy input of 3 to 10 MJ/t or 1 to 3 kWh/t [13]. The data obtained indicates that PEF treatment operates with high efficiency and is able to achieve an equivalent product yield with lower pressure and temperatures.

These studies show that replacing single unit operations with PEF has a beneficial impact on energy production and influences good products. Utilizing the opportunity of heat preservation and recovery will produce economic benefits. Lastly, the usage of non-thermal processing adds energy efficiency for production of vitamin C.

7.3.2 Advantages of Orange juice for biosynthesis of Vitamin C

One of the most popular and prevalent fruit juices in the world is orange juice. This is due to its fresh flavour, especially valued for its highly beneficial high content of vitamin C and natural antioxidants. Vitamin C is an essential nutrient for human and animal consumption. The water-soluble vitamin helps to avoid scurvy and cancer, alleviates the general cold, promotes synthesis of collagen and has a significant role in the process of natural wound healing. Vitamin C can help to enhance the availability and absorption of iron from non-heme sources [2]. The other benefits of orange juice as a source of natural vitamin C is due to its antioxidant properties to stop oxidation of free radicals from damaging the cells. In the food industrial sector, antioxidants are also useful and act as food additives [14]. Vitamin C as the organic compound found to be composed of carbon, hydrogen and oxygen [15].

Furthermore, vitamin C contains other organic compounds such as oxidized, ester and synthetic formed. L-ascorbic acid is the predominant biological sources of vitamin C and dehydroascorbic acid is considered oxidized sources reversibly [2]. Oxidation may cause by several factors including pH, light, heat, oxygen and metal ion. The biological system of human cannot synthesize their own vitamin C as their needed external sources such as diet to supply the cells in the body. Human cells are cannot to perform the final necessary step in vitamin C biosynthesis. The conversion of L-gulono-g-lactone to ascorbic acid is aided by gulonolactone oxidase enzymes as a catalyst to enhance the conversion [2]. The need of vitamin C in human body is to maintain the physiological function. The consumption of vitamin C from diet is one of the requirements to meet the human need as the recommended to take 70mg per day as an adult. The intake of 45mg per day is to ensure the measurable amount of ascorbate is present in the plasma of the most people. This is to supply tissue metabolism to repair at sites of damage.

7.3.3 Advantages of Orange juice in term of vitamin C content

The vitamin C content in orange juice is proven to be the highest compared to the other citrus fruits such as grapefruit, lemon, lime, kaffir lime musk lime [6]. The vitamin C in orange juice remained as the highest content compared to the other citrus fruit using the HPLC method, which is believed to be more accurate, reliable and specific [6].

7.3.4 Advantages of Flavonoids in Orange juice

Flavonoids are a diverse group of phytonutrients found in most fruits and vegetables. Along with the carotenoids, flavonoids are responsible for the vivid colours in fruits and vegetables. By taking or consuming orange juice that contains 272 mg of flavonoids influence the acute improvement of cognitive function and subjective alertness in human health up to 6 hours compared to taking energy drinking without flavonoids content. Flavonoids have some of the health benefits associated with diet such as orange juice. Orange juice is a source of flavonoids and polyphenols. Data collected from epidemiological research and chronic inventions shows that the consumption of fruit juice over a lifetime is able to reduce risk of neuropsychological disease, attenuation of aging-induced cognitive decline, and maintenance of optimal cognitive facilities. [16]

The primary source of flavonoid intake is mainly from the diet. The average daily intake is about 70 mg and 170 mg per day, but it mostly differs depending on the dietary and cultural habits of humans. Citrus fruits are the most popular source of flavonoids. The most important Citrus fruits are oranges, followed by mandarins, grapefruits, lemons, bergamots and limes. Flavonoids with functional activities as a strong antioxidant and radical scavenging activity appear to be able in reducing the risk for certain chronic diseases, and excellent in the prevention of certain cardiovascular disorders and certain types of cancer [17].

7.4 EFFECT OF THE USE HP AND PEF IN THE PRODUCTION OF VITAMIN C AS ANTIOXIDANT FROM ORANGE JUICE ON TODAY SOCIETY

Biotechnology applications nowadays are held in various areas such as in medical, industrial, and agricultural to ease human daily needs and enhance quality of lives. Medical biotechnology that uses cell materials to yield pharmaceutical products capable in treatment and prevention of human diseases are now widely used in industry. The increasing use of biotechnology is becoming most popular as natural alternatives to synthetic medicines in modern society including the production of vitamin C as antioxidant. By using high technology from non-thermal processing technologies of High Pressure (HP) and Electric Potential Fields (PEF) are seen to exert some effect on society as elaborated below.

7.4.1 Lower the risk of disease and improving the healthy lifestyle of community

In recent years, the reported cases of cancer, hypertension and other oxidative stress-related diseases, especially in developing countries, have generally increased. The implications of emerging developments on the expected scale of biotechnology go beyond the factories and testing resources that affect our everyday lives. Diseases are recognized and diagnosed through biotechnology. The avoidance of some forms of cancer through vitamin C. Vitamin C may restrict carcinogenic structures such as nitrosamines, modulate immune response and probably reduce oxidative damage that can contribute to cancer through its antioxidant role. With higher fruit and vegetable intake the risk of most cancers can be minimized because of the high content of vitamin C. It may reduce risk of cardiovascular disease. This composition may be related to the antioxidant content of vitamin C, including an oxidative alteration of low density lipoproteins, and its antioxidant effects [18].

For normal physiological activities, the body needs vitamin C. It helps the human body's synthesis and metabolism. It defends the body from the harmful effects of free radicals, contaminants and toxins as an antioxidant. Vitamin C is very important for various physiological functions in the body as the collagen formation in which it is able to strengthen bone structure, muscle and blood vessels in the body are also affected by vitamin C. It helps to protect cells from free radicals attack. People who are deficient in Vitamin C cannot replace collagen in their body [2,6]. As a result, all types of tissue in the body become damaged and can cause a variety of diseases. According to the Ministry of Health Malaysia, there are studies showing that Vitamin C may be effective in some diseases including flu. Although there is no convincing evidence that Vitamin C can cure the flu, it is one of the popular health supplements that is often a good choice for those with the flu. While we need adequate Vitamin C intake for the body, it is not recommended to be taken in excess.

Vitamin C has been seen and promoted for the prevention or treatment of various health conditions because of its antioxidant capacity and its immune system. The association between fruit intake and cognitive performance has recently been found to be of relevance. In the healthcare sector, vitamin C is rising and gaining popularity due to the bioactive compounds such as carotenoids, antioxidant flavanones, glycosides and other phytochemical compounds. Vitamin C that is rich with flavonoids content shows the strong antioxidants function to reduce the oxidative stress related to cancer and high blood pressure. Besides, antioxidant compounds in orange juice are also used to decrease risk of heart disease, cognitive infarction and ischemic artery disorder [18]. The bioactive compound in the orange juice is commonly lost during the processing of vitamin C by using traditional thermal processing. This is because most of the desired content in products such as bioactive compounds are degraded due to the sensitivity to the heat applied.

By using the HPP and PEF as non-thermal processing technologies, we are able to maintain and preserve the bioactive compound from heat damage. It is proven from the higher performance that can tolerate nutritional value, flavour, texture, scents and thus prolong shelf life. HPP and PEF offer an advantage of minimal heat penetration and heat damage to selective products. Thus, it is important to use HPP and PEF instead of traditional heat treatment for orange juice processing, as it can preserve antioxidant compounds from losses during vitamin C production [9,10]. Living a healthy lifestyle is more than just choosing to eat well and exercise, but also taking care of our body's needs, such as the micronutrients and macronutrients found in our food sources, such as plants and fruits. To generalize, the use of HPP and PEF as processing technologies in the production of vitamin C as antioxidants is worth improving a healthy lifestyle of the human community.

7.4.2 Safe product for the consumer consumption

The essential to safety is the careful handling of experiments. Several years of experience in treating highly dangerous microorganisms has been achieved by microbiologists. Improved design, treatment, method and tests based on biotechnological advances can be used to monitor chemical or product manufacturing faults. Thermal treatment such as pasteurization and sterilization was common and considered to have a spoilage and pathogenic microorganism inactivation ability. Amid this, colour modifications, flavour or even significant nutritional losses are apparent because of these thermal treatments [19]. HP and PEF should be applied alone or together with other preservation methods, for additional advantages. The usage of modern manufacturing techniques can be important because it may result in overall favourable improvements in the actual product qualities. As both are eco-friendly processing methods with fewer environmental effects, HP and PEF are very useful in retaining the ingredients used in producing Vitamin C.

The emerging innovation of new technologies of HPP and PEF in the production of vitamin C as antioxidants from orange juice is remarkable. To produce a good vitamin C product, alteration of processing parameters and conditions must be considered. While some important quality parameters of production vitamin C can be improved by accessing HP and PEF, a better understanding of their impacts on sensory, nutritional, chemical and microbiological properties is still required. In addition, this information will result in good quality products which are desirable to the customer and beneficial to society.

7.4.3 Environmental and co-friendly technology

The potential for improving sustainability in the human lifestyle is now becoming more prominent and much needed for the care and well-being of future generations. Using the HPP and PEF as processing technologies in the production of vitamin C as antioxidants seems promising to improve sustainability in human life today. Environmental technologies aim to protect the environment. In this content HPP and PEF offer ways to minimize the pollution and increase sustainability.

HPP with great advantages and characteristics extends product shelf life, innovation, and competitive advantages, able to maintain high yields and only needs water which is environmentally friendly in the production of vitamin C [20]. This method is in harmony with responsibility towards nature. Another non thermal processing technology which is PEF also has the great advantages that does not involve dewatering or drying, no additional chemical, and no heating thus reducing the operational cost, less energy consumption and less time consuming with high scalability [20].

The greatest distinction between PEF and HPP perception can be seen in the production of vitamin C as an antioxidant from orange juice was only linked to positive effects and innovative emerging environmental technologies. Generally, environmental technology is expensive because it accounts for the environmental costs that are externalized in production processes. However, the perceived benefits are more superior with high enable value return. Thus, a variety of other obstacles cannot limit the acceptance and dissemination of these technologies. These technologies will also make a positive contribution to corporate social responsibility and as a community framework leading by example in environmental awareness. As manufacturers become more environmentally conscious, they are constantly developing alternatives for many prevalent machines and applications.

7.4.4 Job Opportunity for society

Biotechnology is important for a lot of things that can make human lives much more prominent. The field work for both biology and technology are getting advanced leading to the new designed product enriching the lives, making daily days easier and healthier. Vitamin C production as antioxidants may result in more biotechnology careers for new graduates and jobless. From the raw materials or sources with high availability, low cost, less toxicity towards human and animals, sustainable, and degradable to beneficial products enable the production to enhance worldwide attention and result in a surge of commercial interest.

The utilization of HP and HEP as new preservation methods in processing vitamin C from natural resources would probably increase job opportunities for the society as it can compete in the global market. The job opportunity is offered to the society including in the research and development sector of biotechnology, production sector and plantation of raw materials.

7.5 CONCLUSIONS

The application of HP and PEF to produce food-derived vitamin C products have been widely developed to achieve high sensory, nutritional, and microbiological quality. Studies have shown that use in the production of vitamin C or supplements of HP or PEF-treated orange juice can have a positive impact both on their physical, chemical, or microbiological properties and on production outcomes. Because of the preservation of antioxidant doses in the development of vitamin C, the parameters and conditions involved in its processing must be optimised in light of the new challenges imposed by raw materials treated with HP or PEF. The adjustment of processing parameters and conditions depending on each type of product must therefore be taken into consideration. In the proposed process, the efficacy is similar in both configurations (HP and PEF) and both have higher efficacy than that observed with traditional thermal treatment. The concentrated juice also retains its nutritional content that is slightly lost in thermal absorption. Based on these results, it is possible to consider producing high quality concentrated juice, bearing in mind that all technological steps will be carried out sequentially in the final process. In our case, the different treatments performed on orange juice to preserve the antioxidant could have even better results on HP and PEF compared to traditional thermal treatment.

Thermal processes are very effective but may have adverse effects on organoleptic / nutritional components. A variety of new technical methods are under research, primarily focused on the implementation of high-pressure processing as well as pulsed electrical fields, which help maintain the bioactive components of orange juice. The alternative approach is focused on the processing of concentrates, which are primarily generated by vacuum evaporation of water. There are several research on the use of membrane technologies, but it is still under further study. Membrane technologies capable of extracting water without heating are under study for potential industrial applications and that have higher nutritional and organoleptic content juices in the immediate future.

REFERENCES

[1] Carr, A. C., & Vissers, M. (2013). Synthetic or food-derived vitamin C—are they equally bioavailable?. *Nutrients*, *5*(11), 4284-4304.

[2] Yussif, N.M. (2018). Vitamin C. In: LEBlanc, J.G. (Ed.), *Vitamin C - an Update on Current Uses and Functions*. IntechOpen. https://doi.org/10.5772/intechopen.81783.

[3] Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J., & Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian journal of clinical biochemistry*, *28*(4), 314-328.

[4] Mahajan, P. V., Caleb, O. J., Singh, Z., Watkins, C. B., & Geyer, M. (2014). Postharvest treatments of fresh produce. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *372*(2017), 20130309.

[5] Najwa, F. R., & Azrina, A. (2017). Comparison of vitamin C content in citrus fruits by titration and high-performance liquid chromatography (HPLC) methods. *International Food Research Journal*, *24*(2), 726.

[6] Galaverna, G., Silvestro, G. D., Cassano, A., Sforza, S., Dossena, A., Drioli, E., & Marchelli, R. (2008). A new integrated membrane process for the production of concentrated blood orange juice: Effect on bioactive compounds and antioxidant activity. Food Chemistry, 106(3), 1021–1030. doi: 10.1016/j.foodchem.2007.07.018

[7] Galaverna, G., & Dall'Asta, C. (2014). Production Processes of Orange Juice and Effects on Antioxidant Components. Processing and Impact on Antioxidants in Beverages, 203–214. doi: 10.1016/b978-0-12-404738-9.00021-0

[8] Plaza, L., Sánchez-Moreno, C., Elez-Martínez, P., Ancos, B. D., Martín-Belloso, O., & Cano, M. P. (2006). Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. European Food Research and Technology, 223(4), 487–493. doi: 10.1007/s00217-005-0228-2

[9] De Ancos, B., Rodrigo, M. J., Sánchez-Moreno, C., Cano, M. P., & Zacarías, L. (2020). Effect of high-pressure processing applied as pretreatment on carotenoids, flavonoids and vitamin C in juice of the sweet oranges' Navel' and the red-fleshed 'Cara'. *Food Research International*, *132*, 109105.

[10] Zhang, Z. H., Zeng, X. A., Brennan, C. S., Brennan, M., Han, Z., & Xiong, X. Y. (2015). Effects of pulsed electric fields (PEF) on vitamin C and its antioxidant properties. *International Journal of Molecular Sciences*, *16*(10), 24159-24173.

[11] Rodriguez-Gonzalez, O., Buckow, R., Koutchma, T., & Balasubramaniam, V.

M. (2015). Energy requirements for alternative food processing technologies—principles, assumptions, and evaluation of efficiency. *Comprehensive Reviews in Food Science and Food Safety*, *14*(5), 536-554.

[12] Bazhal, M.; Vorobiev, E. Electrical Treatment of apple cossettes for intensifying juice pressing. Journal of the Science of Food and Agriculture 2000, 80, 1668–1674.

[13] Eshtiaghi, M.N.; Knorr, D. 1999, Process for treatment of sugar beet. European Patent No. EP99923708. Filed June 9, 1999; approved March 3, 2001.

[14] Whitney, E. and Rolfes, S.R. 2008. Understanding Nutrition. 11thedn. USA: Thomas Wadsworth.

[15] Chinnici, F., Spinabelli, U., Riponi, C., & Amati, A. (2005). Optimization of the determination of organic acids and sugars in fruit juices by ion-exclusion liquid chromatography. Journal of food composition and analysis, 18(2-3), 121-130.

[16] Macready AL, Kennedy OB, Ellis JA, Williams CM, Spencer JPE, Butler LT (2009) Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. Genes Nutr 4:227–242

[17] Lamport DJ, Dye L, Wightman JD, Lawton CL (2012) The effects of flavonoid and other polyphenol consumption on cognitive performance: a systematic research review of human experimental and epidemiological studies. Nutr Aging 1:5–25

[18] Cassidy A, Rimm EB, O'Reilly ÉJ, Logroscino G, Kay C, Chiuve SE, Rexrode KM (2012) Dietary flavonoids and risk of stroke in women. Stroke 43:946–951

[19] Tahiri, I., Makhlouf, J., Paquin, P., & Fliss, I. (2006). Inactivation of food spoilage bacteria and Escherichia coli O157:H7 in phosphate buffer and orange juice using dynamic high pressure. Food Research International, 39(1), 98e105.

[20] Pereira, R. N., & Vicente, A. A. (2009). Environmental impact of novel thermal and non-thermal technologies in food processing. Food Research International. doi:10.1016/j.foodres.2009.09.013