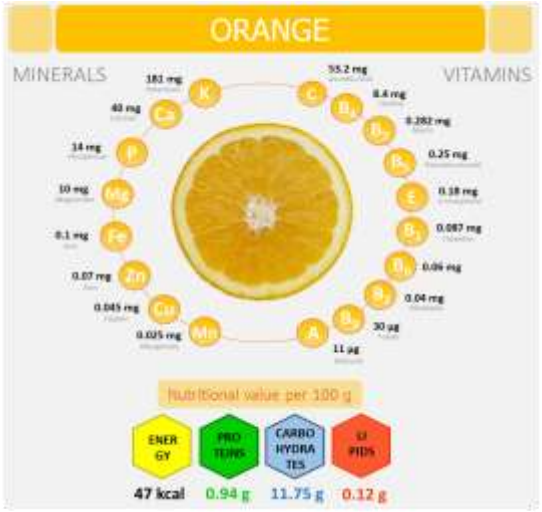
PROPERTIES OF CITRUS FRUIT

- **CITRUS FRUIT** are the first fruit-tree in the world, and one of the most commercialized horticultural crops worldwide, being highly appreciated and demanded for both fresh and juice consumption.
- **ORANGE FRUIT** (*Citrus sinensis*) are an important source of bioactive compounds and phytonutrients such as vitamins A, C, E and folic acid, minerals, flavonoids, phenolic compounds, limonoids, carotenoids, pectins and fiber, among others. *Source[1,2,3]*

- The intake of these **BIOACTIVE COMPOUNDS** through fresh or juice consumption has been related to important **HEALTH-related BENEFITS**, including: *Source[1,2,3,4,5,6]*
  - Antioxidants
  - Anti-inflammatory
  - Reduction of the risk of certain cancers and cardiovascular diseases
  - Hepatoprotectors
  - Obesity control
  - Immune system enhancement



"The moderate consumption provides potential health benefits"

Source[4,5]

"Studies in humans show enhancement of cardiovascular markers, insulin sensibility and body-fat reduction"

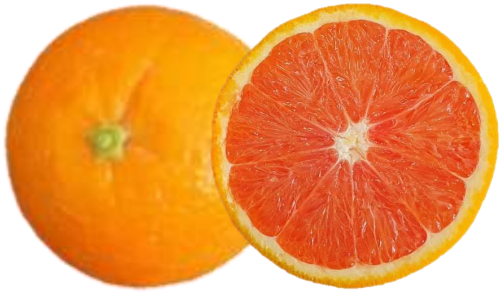
Source[5,6,7,8]

"Oxidative stress plays an important role in the development of human diseases"

Source[3,9,10]

NEW RED-FLESHED ORANGE VARIETIES

- The citrus industry and consumers demand new varieties with new distinguish features and **high added value** related to human nutrition and health.
- **Citrus Rosso** is introducing new red-orange varieties into the market.



- **What are the main characteristics of these varieties?**
- **Why the pulp is red?**
- **What properties these varieties provide compared to the traditional oranges?**

Accumulation of **LYCOPENE** in the pulp

- **Lycopene** is the **CAROTENOID** that provides the reddish and pink color to several fruits, such as tomato and watermelon.
- The accumulation of lycopene in citrus fruit is an unusual feature restricted to only few cultivars of grapefruit, pummelo and sweet orange.

Source[11,12]

What are CAROTENOIDS?

Carotenoids are the pigments responsible for the color of the peel and pulp of citrus fruit. The variability of carotenoids content and composition provides the particular color of the different orange and mandarin varieties.

Source[12,13]

Additionally, carotenoids have two key properties related to human health:

Source[14,15,16,17]

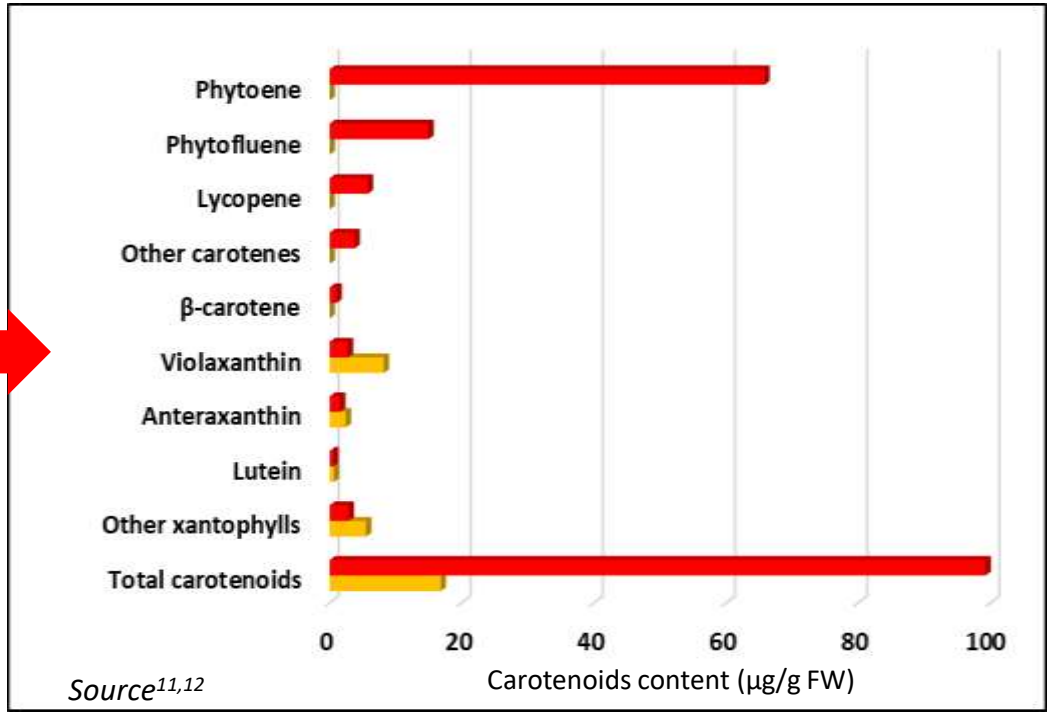
- **ANTIOXIDANTS**
- **VITAMIN A PRECURSORS**

Traditional variety

Red orange variety

The carotenoid content in the pulp of the red oranges is much higher than that of the traditional oranges, and moreover:

- Contains **lycopene**, which is completely absent in ordinary oranges
- Very high amounts of other carotenes, **phytoene** and **phytofluene**



Source[11,12]

What features does LYCOPENE have?

- Due to its chemical structure, lycopene has a **high antioxidant capacity.**
- Numerous epidemiological studies, *in vivo* and *in vitro,* suggest that the regular intake of lycopene has beneficial effects for health, as the reduction of the risk of degenerative diseases.

Source[7,18,19,22,23,24]

POTENTIAL BIOLOGICAL EFFECTS

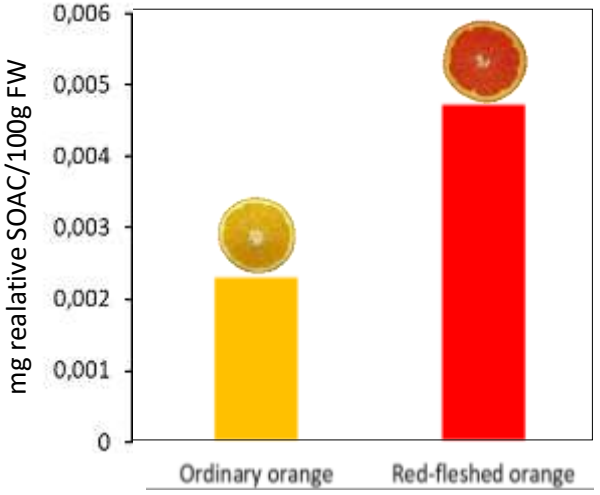
Reduction of:

- Oxidative stress
- Risk of cardiovascular diseases
- Hypertension
- Risk of lung, prostate and stomach cancer
- Cholesterol LDL
- Lipidic oxidation and atherosclerosis

Source[7,18,19,20,21,22,23,24]

In vitro assays

Higher antioxidant capacity than ordinary oranges against "singlet oxygen" radical

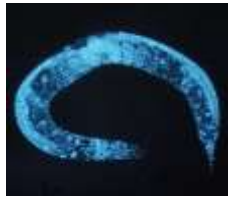


NEW RED-FLESHED ORANGE VARIETIES

in vivo

1 The *C. elegans* worm as a model system for the study of neurodegenerative diseases

- Worms accumulating β-amyloid peptides in muscle cells have been generated.
- Neuromuscular defects (paralysis) and disturbances in certain human-like metabolic functions.



Caenorhabditis elegans

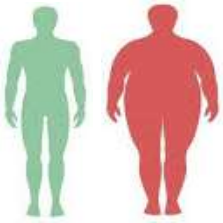
1-10% juice

- ✓ = Lifespan ⇧⇧
- ✓ + Resistance to oxidative stress ⇧⇧
- ✓ - Paralysis Alzheimer's model ⇩⇩

de Oliveira et al., 2019

2 Epidemiological studies have demonstrated that the intake of red-fleshed orange provide relevant beneficial effects for health

Adults
(Normal weight and overweight)



8 weeks:
750 mL of orange juice/day
+ normal diet

- Reduction of total cholesterol
- Reduction of LDL-C
- Enhancement of antioxidant capacity
- Reduction of C-reactive protein (anti-inflammatory processes)

Silveira et al., 2015

Source on unusual carotenes

- **Phytoene** and **phytofluene** are two colourless carotenes which accumulated in very high concentrations in the pulp of the red oranges compared to the traditional oranges.

Content of phytoene and phytofluene in mature red oranges

Carotenoids (µg/g Fresh Weight)	**Peel**	**Pulp**
Phytoene	76 - 100	65 - 150
Phytofluene	0 - 3	15 - 21

- Recent studies indicate that these carotenes may have relevant beneficial properties for health and as nutricosmetic:

  - Protection against oxidative stress
  - Enhancement of anti-inflammatory response
  - Reduction of cholesterol in plasma
  - Positive effects related to breast and prostate cancer
  - Protection against ultraviolet radiation (UV)

Source[25,26,27,28,29]

FLAVONOIDS

- Flavonoids are a family of natural compounds which occurred in relatively high concentrations in citrus fruits. They are widely recognized by their healthy properties, including:
  - Antioxidants
  - Anti-inflammatory
  - Cardiovascular
  - Anticancer

  Source[1,2,3,4,5,31,32,34]

- No differences in the content of the main flavonoids between mature fruits of the red-fleshed and ordinary orange varieties.

Flavonoids (mg/100g)	Ordinary variety	Red-fleshed variety
Rutin	2.63±0.17	2.69±0.60
Eriocitrin	0.98±0.09	1.08±0.13
Narirutin	7.58±1.05	9.08±1.90
Naringin	0.28±0.0.03	0.30±0.07
Hesperidin	34.62±6.78	45.19±3.55
Dydimin	0.85±0.13	1.17±0.26

The **flavonoids** content is **similar** to that of ordinary varieties and therefore, the **healthy benefits** remain **intact** in the red-fleshed orange varieties

Internal quality and other features

- In the red-fleshed oranges **no significant differences** in other quality parameters, such as ºBrix, acidity or vitamin C were observed. Therefore, their characteristics are similar than those of traditional oranges.

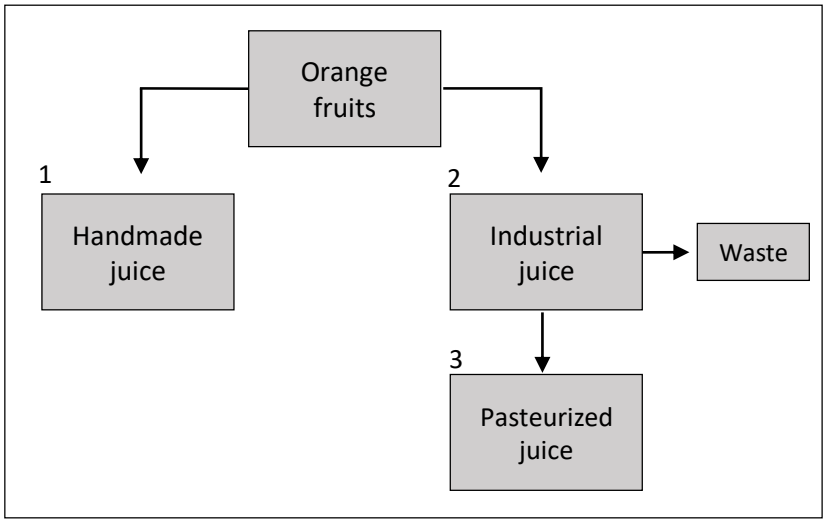
Internal quality	Traditional variety	Red-fleshed variety
Soluble solids (ºBrix)	11.35 ± 0.21	11.25 ± 09
Acidity (mg citric acid/100 ml)	0.86 ± 0.05	0.87 ± 0.03
Maturity index	13.20 ± 0.03	12.93 ± 0.09
Vitamin C (mg/100 g FW)	46.14 ± 3.89	45.16 ± 1.41

- Studies performed by the research group of IATA-CSIC, have shown that **total carotenoids content in red-fleshed oranges is at least 5 times higher** than that of traditional orange varieties.

Red-fleshed oranges provide an additional contribution in antioxidant carotenoids over traditional varieties and their consumption may have an added healthy value

RED-FLESHED ORANGE JUICES

- ***CITRUS ROSSO*** in collaboration with the **INSTITUTE of AGROCHEMISTRY and FOOD TECHNOLOGY** (IATA-CSIC) is working on the development of red-fleshed orange juices and investigating about their industrial and nutraceutic aptitude.
- One of the main goals is to evaluate the content and composition of **bioactive compounds** and **antioxidant activity** of the novel red-fleshed orange juices compared to that of ordinary orange juices.



Type of orange juice analyzed

1. **Handmade:** Fresh juice extracted by *Citromatic* handmade squeezer
2. **Industrial**: Fresh juice extracted by industrial extractor
3. **Pasteurized**: Orange juice pasteurized at 85ºC

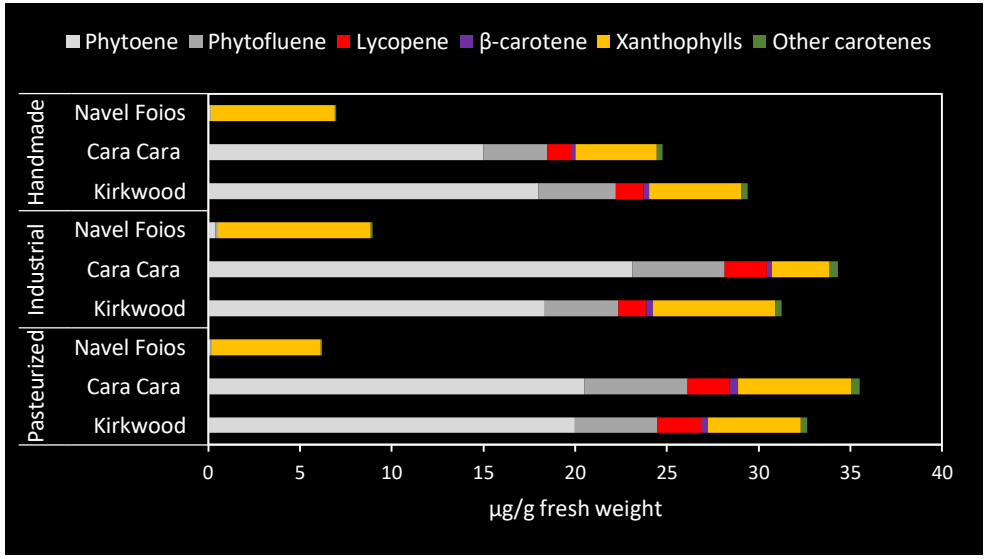
Quality parameters of the red-fleshed orange juices

Parameter	Juice	Navel	Cara Cara	Kirkwood
Soluble solids (ºBrix)	Handmade	11.45 ± 0.07	9.97 ± 0.06	11.17± 0.06
	Industrial	11.33 ± 0.06	10.87 ± 0.12	11.56 ± 0.06
	Pasteurized	10.67 ± 0.06	10.67 ± 0.06	10.7 ± 0.20
Acidity (mg CA/100 ml)	Handmade	0.82 ± 0.05	0.70 ± 0.06	0.91 ± 0.06
	Industrial	0.86 ± 0.14	0.79 ± 0.10	0.86 ± 0.06
	Pasteurized	0.82 ± 0.08	0.89 ± 0.15	0.75 ± 0.02
Maturity index	Handmade	14.08 ± 0.94	14.36 ± 1.04	12.35 ± 0.87
	Industrial	13.39 ± 2.01	13.95 ± 1.65	13.46 ± 1.14
	Pasteurized	13.02 ± 1.10	11.98 ± 1.62	14.21 ± 0.45

No significant differences in maturity index among the juices of the different orange varieties

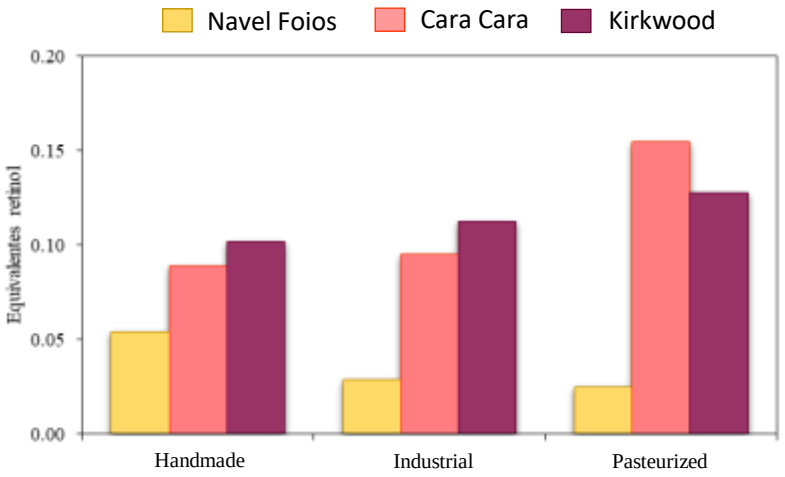
RED-FLESHED ORANGE JUICES

Carotenoids



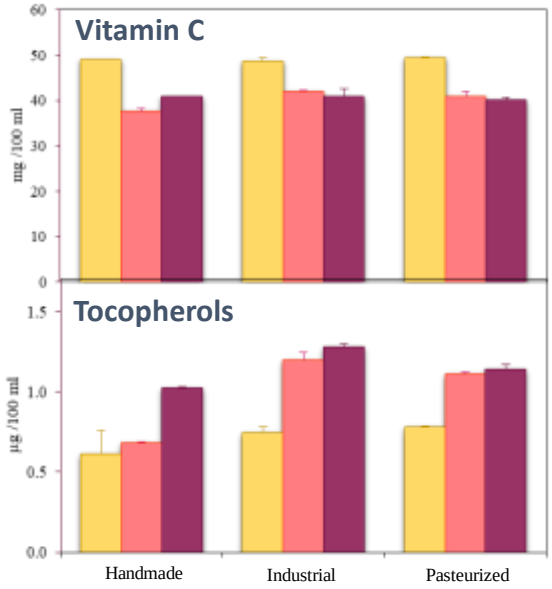
Xanthophylls: family of carotenoids that provide yellow and orange coloration to citrus fruits.

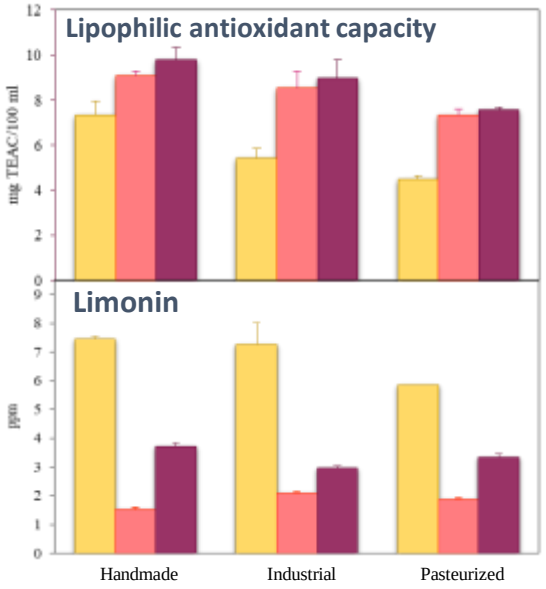
Pro-vitamin A activity



- The red-fleshed orange juices contain between 4 and 6 times higher total carotenoid content.
- Apart from lycopene, the red-fleshed orane juices contain colorless carotenes, such as phytoene and phytofluene, accounting around 60% of the total carotenoids detected. Interestingly, these carotenoids are in very low concentrations in ordinary oranges.
- The red-fleshed orange juices contain higher levels of β-carotene, precursor of retinol and provide a higher source of vitamin A.

RED-FLESHED ORANGE JUICES





In the red-fleshed orange juices:

- The concentration of vitamin C is slightly lower than that of the ordinary varieties.
- The tocopherols content (Vitamin E) is similar or slightly higher than that of the ordinary varieties.

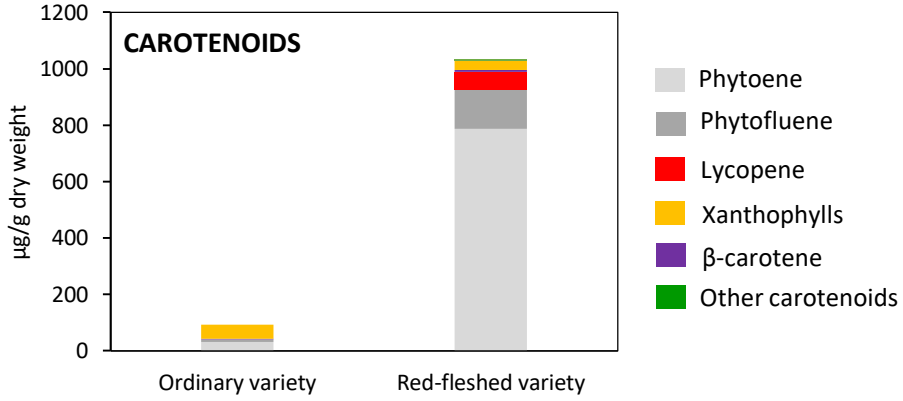
In the red-fleshed orange juices:

- Higher lipophilic antioxidant capacity due to their large carotenoids amounts.
- Minor levels of limonin than Navel Foios at similar maturation index.

The results indicate that **the new red-fleshed orange juices** provide a **higher carotenoid content**, a **higher antioxidant capacity** and also, they contain **reduced levels of limonin**. On the whole, these kind of orange juices show great potential for both **nutritional** and **industrial** level.

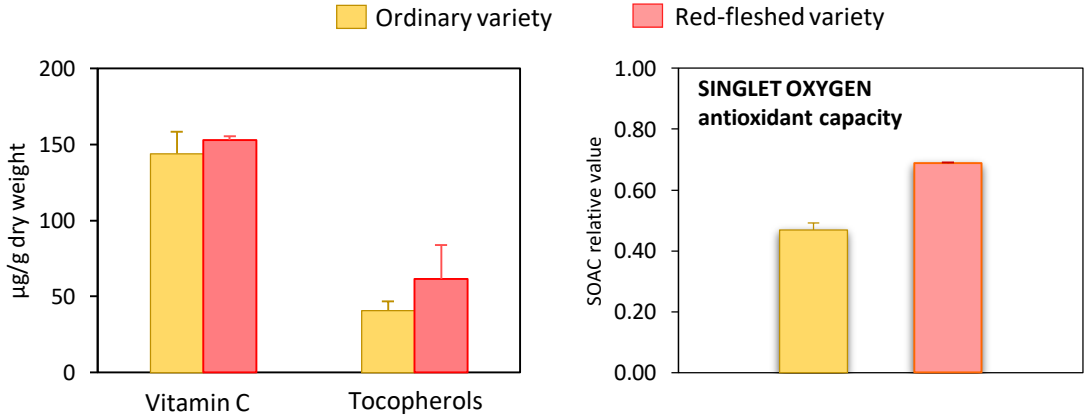
RED-FLESHED ORANGE BY-PRODUCT

- The fruit **waste** generated during the juice extraction is one of the biggest downsides of the citrus juice industry.
- ***CITRUS ROSSO*** works on the revaluation of citrus juice by-products.
- The **red-fleshed oranges juice by-products** represents a **enriched source of carotenoids** with high potential **to be reused** in the food and feed industries.



Ordinary variety by-products

Red-fleshed variety by-products



References

1. Gironés-Vilaplana A, Moreno DA and García-Viguera C (2014) Phytochemistry and biological activity of Spanish Citrus fruits. *Food and Function* 5: 764-772.
2. Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O, Xiao C, Lu C and Liu Y (2015) Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. *Chemistry Central Journal* 9: 68.
3. Zou Z, Xi W, Hu Y, Nie C and Zhiquin Z (2016) Antioxidant activity of Citrus fruits. *Food Chemistry* 196: 885-896.
4. Ma G, Zhang L, Sugiura M and Kato M (2020) Citrus and health. In: Talón M, Caruso M and Fred Gmitter FG (eds) The Genus Citrus. Cambridge: Woodhead Publishing, pp, 495-508.
5. Turner T and Burri BJ (2013) Potential Nutritional benefits of current citrus consumption. *Agriculture* 3: 170-187.
6. Cirmi S, Maugeri A, Ferlazzo N, Gangemi S, Calapai G, Schumacher U and Navarra M (2017), Anticancer potential of citrus juices and their extracts: a systematic review of both preclinical and clinical studies. *Frontiers* 8:420.
7. Silveira JQ, Grace KZS, Cesar D and Cesar TB (2015) Red-fleshed sweet orange juice improves the risk factors for metabolic syndrome. International Journal of Food Sciences and Nutrition 66(7): 830-836.
8. Rampersaud GC and Valim MF (2017) 100% citrus juice: Nutritional contribution, dietary benefits, and association with anthropometric measures. *Critical Reviews in Food Science and Nutrition* 57(1): 129-140.
9. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN and Lakshminarasaiah U (2014). Antioxidants and human diseases. *Clinica Chimica Acta*, 436: 332–347.
10. Tan BL and Norhaizan ME (2019). Carotenoids: How effective are they to prevent age-related diseases. Molecules 24: 1801.
11. Alquezar B, Rodrigo MJ and Zacarías L (2008) Regulation of carotenoid biosynthesis during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochemistry* 69: 1997–2007.
12. Zacarias J (2017). Caracterización fisiológica y molecular del metabolismo de carotenoides en dos variedades de naranja (Citrus sinensis) de pulpa roja. Máster Universitario en Calidad y Seguridad Alimentaria, 8ª Edición. Departamento de Medicina Preventiva i Salut Pública, Ciències de l'Alimentació, Toxicologia i Medicina Legal, Universidad de Valencia, España.
13. Alquézar B, Rodrigo MJ and Zacarías L (2008b) Carotenoid biosynthesis and their regulation in citrus fruits. *Tree for Science Biotechnology* 2: 23-35.
14. Rodrigo MJ, Alquézar B, Alós E, Lado J and Zacarias L (2013a) Biochemical bases and molecular regulation of pigmentation in the peel of Citrus fruit. *Scientia Horticulturae* 163: 46–62.
15. Amengual J (2019). Bioactive Properties of Carotenoids in Human Health. *Nutrients* 11: 2388.
16. Berman J, Zorrilla-López U, Farré G, Zhu C, Sandmann G, Twyman RM, Teresa Capell T and Christou P (2014). Nutritionally important carotenoids as consumer products. *Phytochemistry Reviews* in press (doi: 10.1007/s11101-014-9373-1).
17. Eggersdorfer M and Wyss A (2018). Carotenoids in human nutrition and health. *Archives of Biochemistry and Biophysics* 652: 18–26.
18. Rao A and Rao LG (2007) Carotenoids and human health. *Pharmacological Research* 55: 207–216.
19. Bailey JR (2015) Lycopene: Food sources potential role in human health and antioxidant effects. New York: Nova Publishers.
20. Chen J, Song Y and Zhang L (2013) Effect of lycopene supplementation on oxidative stress: an exploratory systematic review and meta-analysis of randomized controlled trials. *Journal of Medicinal Food* 16(5): 361–374.
21. Basu A and Imrhan V (2007) Tomatoes versus lycopene in oxidative stress and carcinogenesis: conclusions from clinical trials. *European Journal of Clinical Nutrition* 61: 295–303.
22. Chen W, Mao L, Xing H, Xu L, Fu X, Huang L, Huang D, Pu Z and Li Q (2008) Lycopene attenuates $A\beta_{1-42}$ secretion and its toxicity in human cell and Caenorhabditis elegans models of Alzheimer disease. *Neuroscience Letters* 608: 28–33.
23. De Oliveira R, Orlando Muñoz C, Carmona L, Peña L and de Paula Oliveira R (2019) Pasteurized orange juice rich in carotenoids protects *Caenorhabditis elegans* against oxidative stress and β-Amyloid toxicity through direct and indirect mechanisms. *Oxidative Medicine and Cellular Longevity* 5046280.
24. Mein JR, Lian F and Wang XD (2008) Biological activity of lycopene metabolites: implications for cancer prevention. *Nutrition Reviews* 66(12): 667–683.
25. Wang XD (2012) Lycopene metabolism and its biological significance. *American Journal of Clinical Nutrition* 96: 1214S–1122S.
26. Engelmann NJ, Clinton SK and Erdman Jr JW (2011) Nutritional aspects of phytoene and phytofluene, carotenoid precursors to lycopene. *American Society for Nutrition* 2: 51–61.
27. Meléndez-Martínez AJ, Mapelli-Brahm P and Stinco CM (2018) The colourless carotenoids phytoene and phytofluene: from dietary sources to their usefulness for the functional foods and nutricosmetics industries. *Journal of Food Composition and Analysis* 68: 91–103.
28. Meléndez-Martínez AJ, Mapelli-Brahm P, Benítez-González A and Stinco CM (2015) A comprehensive review on the colorless carotenoids phytoene and phytofluene. *Archives of Biochemistry and Biophysics* 572: 188–200.
29. Meléndez-Martínez AJ, Stinco CM and Mapelli-Brahm P (2019) Skin carotenoids in public health and nutricosmetics: the emerging roles and applications of the UV radiation-absorbing colourless carotenoids phytoene and phytofluene. *Nutrients* 11:1093.
30. Aust O, Stahl W, Sies H, Tronnier H and Heinrich U (2005) Supplementation with tomato-based products increases lycopene, phytofluene, and phytoene levels in human serum and protects against UV-light-induced erythema. *International Journal for Vitamin and Nutrition Research* 75:54–60.
31. De Ancos, B., Cilla, A., Barberá, R., Sánchez-Moreno, C., & Cano, M. P. (2017). Influence of orange cultivar and mandarin postharvest storage on polyphenols, ascorbic acid and antioxidante activity during gastrointestinal digestion. Food Chemistry, 225, 114–124-
32. Panche AN, Diwan AD, Chandra SR (2016) . Flavonoids: an overview. *Journal of Nutritional Science* vol. 5, e47, page 1 of 15.
33. Lado J, Gambetta G, Zacarías L (2018). Key determinants of citrus fruit quality: Metabolites and main changes during maturation. *Scientia Horticulturae 233:238-248.*
34. Ma G, Zhang L, Sugiura M, Kato M (2020). Chapter 23: Citrus and Health. The Genus Citrus. 1st Edition. M. Talon, M. Caruso and F. Gmitter, jr. Eds. Elsevier I (2020). Pp 245-269; ISBN: 9780128121634

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