HARNESSING NATURAL VITAMIN C

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Abstract

Consumer demand is driving greater access to natural molecules in their finished products. In 2021, we leverage our expanded knowledge and concentration breakthroughs achieved through Australian innovation in Cellular Extraction processes as we discover new formats to work with the highly unstable natural vitamin C molecule from the Kakadu Plum, the A(ustralian)-beauty hero ingredient championed by Australian brands, sourced from Indigenous supply chains and achieving global popularity. However, confusing misinformation about natural (biological ascorbic acid) versus naturally derived/synthetic/purified vitamin C (ascorbic acid) distracts form the discoveries and significance of maintaining activity for extended periods and breakthrough in concentrations.

There is growing recognition in the beauty industry of the importance in a holistic approach of topical and ingestible support, and in this scientific review we focus upon oral and topical applications of vitamin C (ascorbic acid and ascorbates) and present some of the key scientific evidence both from history and from recent years. It is discussed that while both natural and synthetic vitamin C are absolutely identical at the molecular level, there are further considerations around natural versus synthetic vitamin C that are relevant to health and beauty.

We illustrate how the body maintains blood levels of vitamin C through oral applications and discuss skin absorption from topical applications, with a critical review on some of the synthetic derivatives that have been created by chemical companies. We briefly recount the history of vitamin C discoveries from the widely known accounts of scurvy (scorbutus) which plagued humanity for centuries, to two Nobel Prizes related to the discovery of vitamin C in the 1930s, and up to the most recent research including world leaders from the National Institutes of Health.

There are many claims surrounding vitamin C that are based on anecdotal evidence, or in some cases no evidence at all, that have simply not been substantiated by science. Drawing from a body of scientific literature that spans approximately 40,000 studies we provide factual and fundamental aspects of understanding on vitamin C's functionality and stability. This includes a brief overview of the chemistry of vitamin C in both its reduced and oxidised forms, and we provide the world's leading resources for accurate information on vitamin C so that these easily accessible public resources are more widely recognised.

Vitamin C science largely centres around the antioxidant properties of vitamin C and how it is central to the human body's own antioxidant system based on glutathione, but also how it acts as a co-factor for a number of essential enzymes and how this is critical for both health and beauty. It is not widely understood that vitamin C is regenerated in the blood and tissues of the body once it has been oxidised and we discuss multiple mechanisms for this. We show the unequal distribution of vitamin C throughout the body, from the high demand for vitamin C in the brain, through the medium demand in critical organs, and to the low levels that are found in the skin.

Vitamin C chemistry is outlined in the context of its implications for topical applications. This includes skin absorption and formulation stability for vitamin C and its derivatives. We also address some myths around vitamin C ingredients that are used in topical formulations, and briefly discuss the industry.

Finally, we discuss how Native Extracts' Cellular Extraction process is able to harness natural vitamin C from plant sources like the Kakadu Plum, which is the highest known concentration of vitamin C in nature, and further how Native Extracts has successfully maintained the stability of vitamin C in aqueous solutions for extended product shelf life for over two years.

A Brief Introduction to Vitamin C

In the early days, for hundreds of years the condition of scorbutus was known. Scorbutus is what we today call scurvy which most people know is a severe vitamin C deficiency. Scurvy was not a particularly common ailment until the era of great sea voyages (from around the 1400s onwards) where in pursuit of exploration and colonialization these voyages could last many months. At this point scurvy became quite a serious and common problem. It was known that certain foods could cure patients of scurvy and the most commonly known tales around this relate to citrus. Whilst other foods, including things as simple as salted horse meat, could also address scurvy, citrus was more effective and remained stable for longer on extended voyages than equally effective foods like tomatoes.

Whilst most animals do produce their own vitamin C (not most primates however), the best sources of vitamin C remain fruits and vegetables. For example, citrus and tomatoes have around 50mg/100g, broccoli and kiwifruits have around double this and capsicums have around triple. Then there are more exotic food sources from rose hip (~400mg/100g) to the undisputed leader in terms of vitamin C content, the Kakadu Plum (recorded as high as 7,000mg/100g). It takes a month or two of vitamin C deprivation to manifest as the symptoms for scurvy. This is because the kidneys are very efficient at retaining vitamin C at low levels via specific protein transporters that recycle vitamin C back into the bloodstream, although these protein transporters can become saturated at high intake levels.

The early symptoms of scurvy are largely related to a lost capacity to properly form the collagen matrix which requires hydroxylase enzymes (for which vitamin C is an essential co-factor). Collagen is required for blood vessels and capillaries, so these lose their integrity and many blood vessels and capillaries will start to leak and bleed as the connective tissues break down. This also pertains to other tissues where collagen is critical (e.g. bone, see below). Eventually the patient will suffer from mood disorders and neuropathy as vitamin C is also critical for the generation of certain neurotransmitters, and sleep disorders can also develop.

The first experiment with some degree of proper controls was performed in the mid-1700s by naval officer James Lind. Though this provided some proper data around the treatment of scurvy it would be almost another 200 years before vitamin C was properly discovered and named ascorbic acid. The search was on for a biological factor known as the anti-scorbutic factor or the anti-scorbic factor. This is from where the name ascorbic acid was derived (a-scorbic acid). Vitamin C was first isolated in the 1930s and shortly after its chemical structure and chemical synthesis were achieved. In 1937 the Nobel Prize in Physiology or Medicine was awarded to Hungarian scientist Albert Szent-Györgyi which included his discovery of ascorbic acid as vitamin C (https://www.nobelprize.org/prizes/medicine/1937/summary/) although American scientist Charles King is also credited as a co-discoverer. In the same year the Nobel Prize in Chemistry was shared between two scientists, one of whom is Sir Norman Haworth (although it would be another ten years later before he would be knighted to be a 'sir'), for his work on the chemistry of vitamin C, identifying its chemical structure and also discovering the first successful method for chemical synthesis (https://www.nobelprize.org/prizes/chemistry/1937/summary/).

Technically vitamin C's initial chemical name was reported by Sir Norman Haworth as L-hexuronic acid, and later as two of the most prominent leaders in the field at the time Szent-Györgyi and Haworth agreed that it should be called L-ascorbic acid as the technical chemical name, from which the common name ascorbic acid derives (hence these are all synonyms). It should also be noted at the outset that the ascorbate ions are equally as active as ascorbic acid and in fact, far more dominant at physiological pH.

Since this time just prior to World War II, scientific research into vitamin C has never showed any signs of slowing down and has only accelerated. Today there are approximately 40,000 scientific studies, peer-reviewed, published, and available on vitamin C.

Vitamin C Nutrition Science – A Historical Perspective

In developing this story, we will present data that addresses the nutrition science of vitamin C in the diet and from supplementation, as well as vitamin C nutrient science for topical applications.

When exploring a new field of scientific investigation or to facilitate communication of such science to the uninitiated a good foundation can often be established by presenting a visual timeline of the scientific studies and events that have come before. These are presented below in figure 1 and figure 2.

Following on from the early discoveries in the 1930s, vitamin C was thrust back into the spotlight in the 1970s and 1980s by Linus Pauling. At the time the number of scientific studies on vitamin C was approaching ~10,000 (i.e. about a quarter of the scientific volume that we have today). Linus Pauling was a champion for vitamin C and he formed the Linus Pauling Institute, which still exists today although it has moved from California to Oregon State University. Linus Pauling was a prolific scientist with over 1,200 publications in his lifetime, and he won the Nobel Prize in Chemistry in 1954 for his work on chemical bonds (https://www.nobelprize.org/prizes/chemistry/1954/summary/). His worked paved the way for much of the modern science around complex molecules including the discovery of the structure of DNA by James Watson, Francis Crick, and Rosalind Franklin.

In the 1970s/80s Linus Pauling made many claims around vitamin C, including in relation to the prevention of illnesses and the treatment of cancer. This was highly controversial and was highly contested, most notably by the Mayo Clinic. This led to a fierce debate, and it inevitably damaged Linus Pauling's reputation somewhat and also led to some belief that whilst the essential status of vitamin C to prevent deficiencies was undeniable, that the potential of vitamin C for other applications was overstated.

In Linus Pauling's defence, the scientific tools did not really exist at the time to properly prove or disprove many of the hypotheses that had been presented. Since the rapid expansion of molecular tools in the scientific toolkit in recent decades, some of these questions are being revisited and as mentioned previously, vitamin C science is only accelerating and showing no signs of slowing down. Currently much of the work in vitamin C science is led by the National Institutes of Health (USA) with the most published world-leader in the field being Professor Mark Levine, however there are many international collaborations amongst scientific research groups who work collectively to solve both the fundamental and applied science of vitamin C. In figure 3 below, a schematic shows the categories of various scientific disciplines in which vitamin C research is currently classified.

For those seeking further information on vitamin C science, valuable resources for the most reputable and evidence-based information providers include:

- National Institutes of Health, Office of Dietary Supplements (USA)
  https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/
- National Institutes of Health, National Library of Medicine, National Center for Biotechnology Information, PubChem
  https://pubchem.ncbi.nlm.nih.gov/compound/54670067
- Cochrane Collaboration (UK)
  https://www.cochrane.org/
- Oregon State University, Linus Pauling Institute, Micronutrient Information Center (USA)
  https://lpi.oregonstate.edu/mic/vitamins/vitamin-c

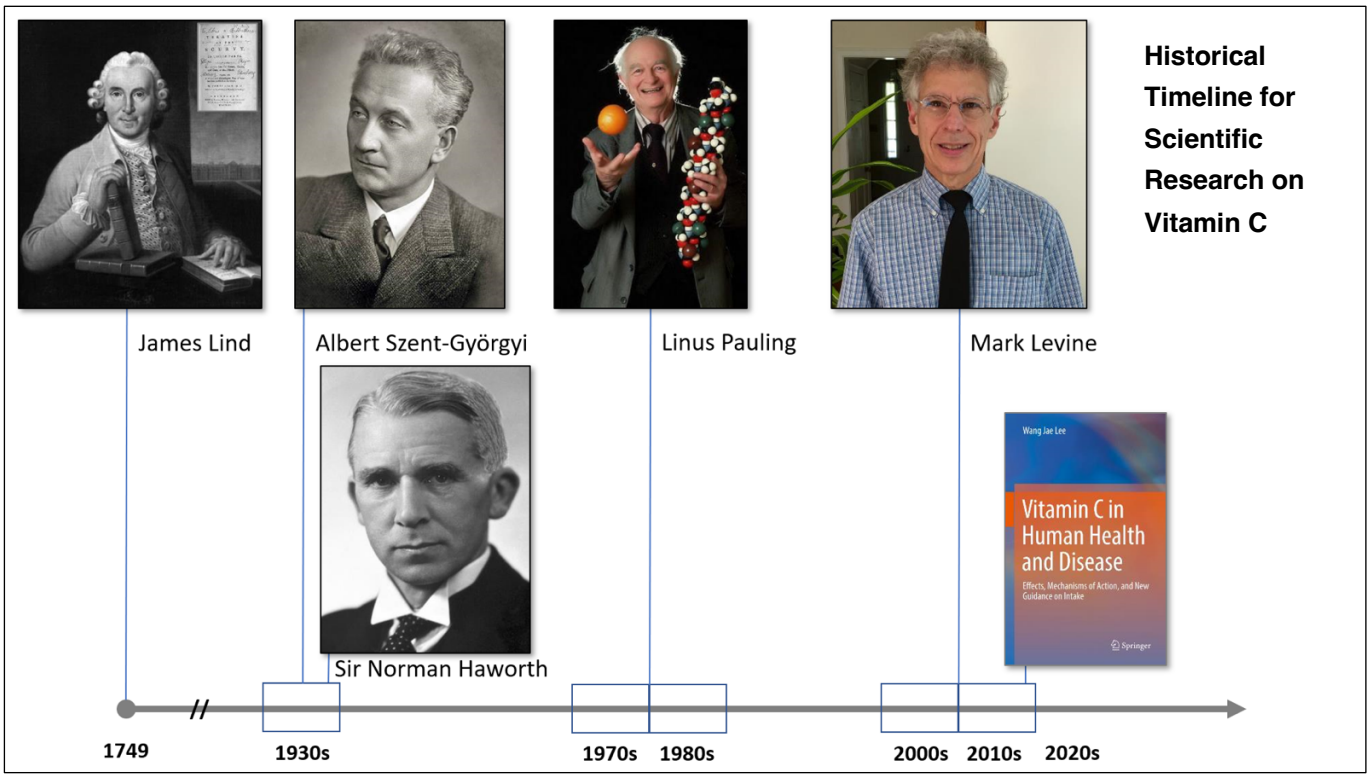


Figure 1: Visual timeline of significant events in the historical development of vitamin C science. This includes early experimentation in the navy by James Lind in the 1700s, the central discoveries of Nobel Prize Winners Albert Szent-Györgyi and Sir Norman Haworth, the promotion of vitamin C in the 1970s/1980s by Linus Pauling, in more modern times the continued research on vitamin C which includes Professor Mark Levine who is currently the most published scientist in the world on vitamin C, and the fact that to this day there are still books being published on vitamin C science.

Number of Research Papers & Reviews on vitamin C – Total (1900 – 2019)

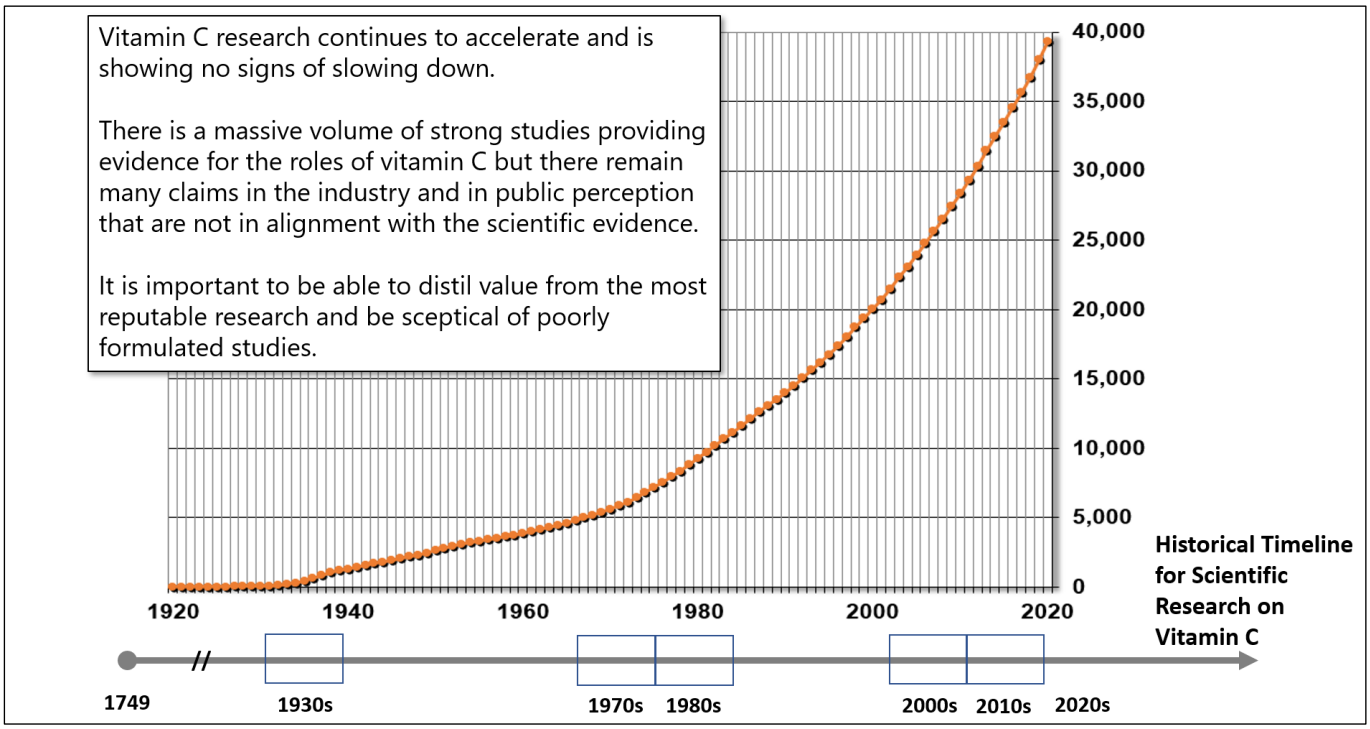
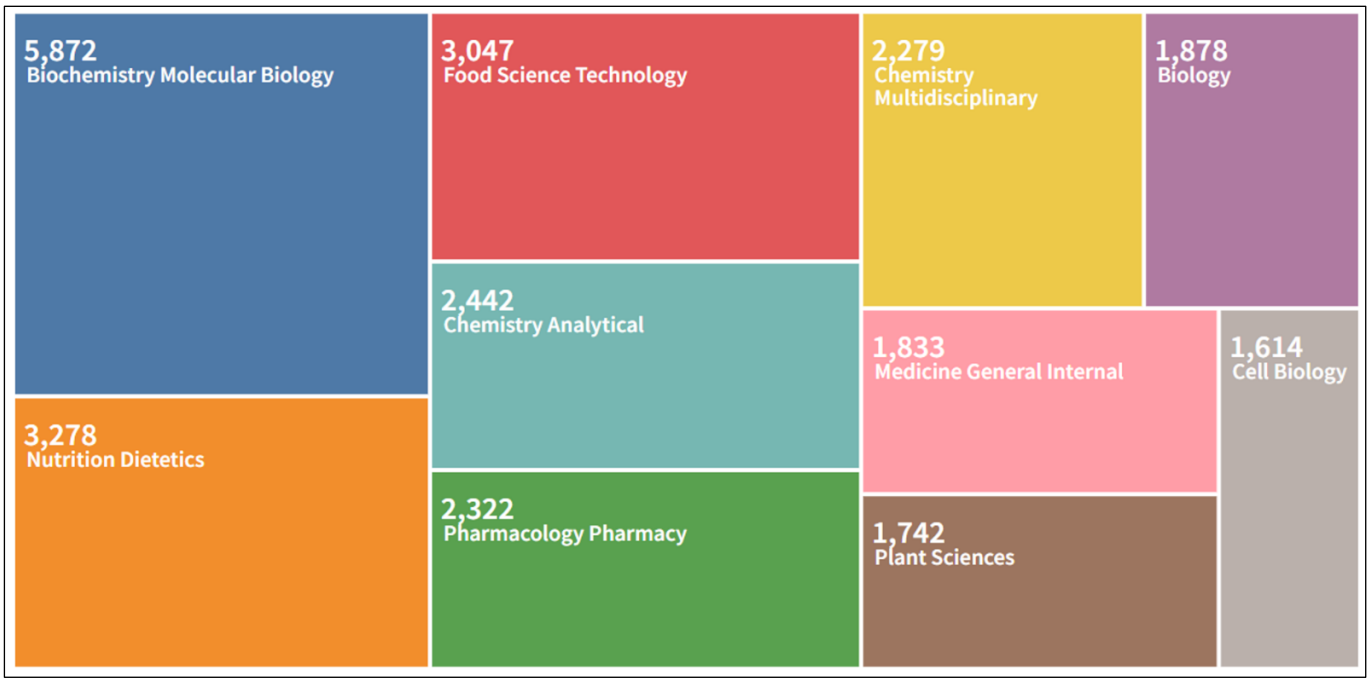


Figure 2: Visual timeline of the volume of scientific studies that have been performed over the last century.

Vitmain C Research is Occuring Across Multiple Scientific Disiplines



It is inadvisable to trust claims made by those who cannot demonstrate multi-disiplinary scientific understandings.

Figure 3: Scientific disciplines that primary vitamin C research is generally classified into. In this schematic, the disciplines most relevant to the cosmetics industry are (1) biochemistry and molecular biology, (2) chemistry analytical, (3) chemistry multidisciplinary, and (4) cell biology.

Vitamin C Biochemistry

Now that important background on vitamin C has been established the focus will shift to the fundamental and applied science of vitamin C functionality which is predominantly a function of its biochemistry. Vitamin C biochemistry is very relevant to both oral and topical applications.

Vitamin C (ascorbic acid and ascorbate ions) is most commonly thought of by the layperson as an antioxidant. While this is true it is important to emphasise that vitamin C has two primary roles:

- An anti-oxidant (and a pro-oxidant) for redox reactions, and;
- A co-factor for multiple critical reactions in the body (especially via hydroxylase enzymes);
- Iron absorption and formation of ferritin;
- Formation of tetrahydrofolate (methyl donor for DNA/AA metabolism);
- Fibroblasts and connective tissue;
- Osteoblasts and bone;
- Hydroxylation reactions (hydroxylase enzymes);
    - Collagen synthesis – hydroxylation of proline and lysine residues, examples:
        - Type 1 skin and bone;
        - Type 2 cartilage;
        - Type 3 reticular fibres, arteries;
        - Type 4 basement membrane;
        - Type 5 hair;
    - Hydroxylation of dopamine to nor-epinephrine;
    - Hydroxylation of tryptophan to serotonin;
    - Hydroxylation of tyrosine to homogentisic acid.

Hence it is very clear that despite its most common reputation as an antioxidant, that in fact a great deal of vitamin C's functions relate to important biochemical processes and not to its role as an antioxidant. This is exactly why scurvy is such a ruinous disease state, as the body gradually begins to fall apart, and neurological disorders ensue.

In this section the focus will be on basic biochemistry of vitamin C and its role in redox reactions, and the topic of vitamin C acting as a co-factor will follow as its own dedicated section.

Basic Biochemistry of Vitamin C – Ionisation and Oxidation

Starting with the basics of vitamin C biochemistry it is important to make clear at the outset that ionisation and oxidation are very different effects in chemistry and biochemistry. Very dramatically different.

However, it has become clear from working with industry that not only is there some confusion around these concepts, but that some bad actors are spreading false and misleading information while they claim to be experts. This is somewhat puzzling given how easy it is to fact check these false claims and myths, so their motivation is a mystery. Either these people are doing this deliberately to misdirect and self-promote (in which case they truly are bad actors), or they are simply lacking basic levels of scientific literacy, which is quite okay, but then don't claim to be an expert.

Ionisation is very simple, while oxidation can be quite complicated. There is no need for formulating chemists to understand oxidation to the deepest levels of complexity and here the description will be kept to the essentials, and for those seeking deeper understanding scientific references are provided.

Ionisation

Starting with ionisation. It is as simple as pH, it really is. Ascorbic acid is a diprotic acid, meaning that it can donate two hydrogen ions (protons), so it has three states which is illustrated in figure 4. The higher the concentration of hydrogen ions, the greater is the pressure for hydrogen ions to remain bonded to the ascorbic acid, and as the concentration of hydrogen ions in the solution decreases, the more likely it is that the ascorbic acid will lose its hydrogen ions. The points of balance between the different forms (i.e. equilibrium between different ionic states) are denoted by pKa values, which vary slightly based on temperature due to Le Châtelier's Principle. For example, the PubChem resource from the National Center for Biotechnology Information (NCBI) states the pKa value as 4.7, but this higher value is because they are using a 10°C temperature reference.

pH Dependent Forms of vitamin C

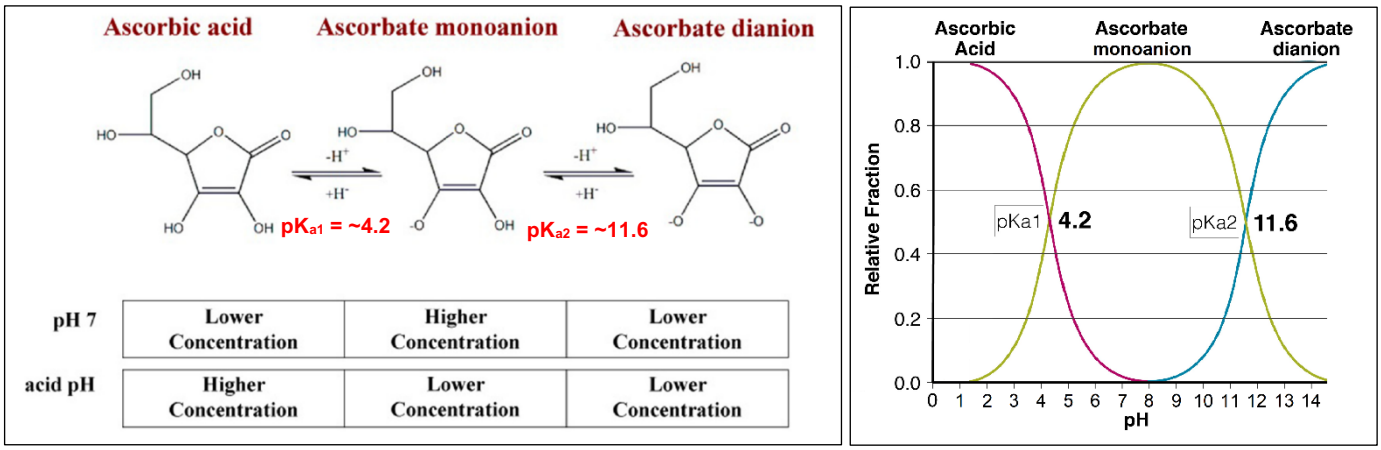


Figure 4: LEFT: pH-Dependent Forms of vitamin C (i.e. Ascorbic acid and its ions). Between pH 6 and 9 most vitamin C will be present as the ascorbate ion (mono-anion). At physiological pH or pH7, then ~99% of vitamin C will be present as the ascorbate ion (mono-anion). Within certain cell compartments where acidity is high and pH values are low, then ascorbic acid will be at higher levels, this is also the case for skincare formulations that have low pH, e.g. pH = 3-4. Ascorbate dianion is quite rare at physiological pH ranges and at neutral pH its concentration will be only 0.005%. pKa1 is the pH at which point ascorbic acid and ascorbate monoanion levels are equal, and pKa2 is the pH where ascorbate monoanion and ascorbate dianion are equal. Source: Figueroa-Méndez & Rivas-Arancibia 2015. RIGHT: Ascorbic acid pKa graph showing the distribution of molecular species and different pH levels, and the two pKa values. Source: Stephens & Minnikin 2021.

Oxidation and Anti-Oxidant Capacity

When considering the antioxidant capacity of vitamin C the discussion focuses on vitamin C's role as an electron donor. For oxidation there is complex molecular chemistry which is outlined in figure 5 below. Vitamin C can have two primary oxidation states, and as is clear from figure 5 the second level of oxidation has been reported to have multiple possible molecular configurations for dehydroascorbate (DHA). For most practical purposes in industry it is not necessary to understand atomic chemistry and it can be generally accepted that DHA1 (see figure 5) enables sufficient understanding of the antioxidant biochemistry processes.

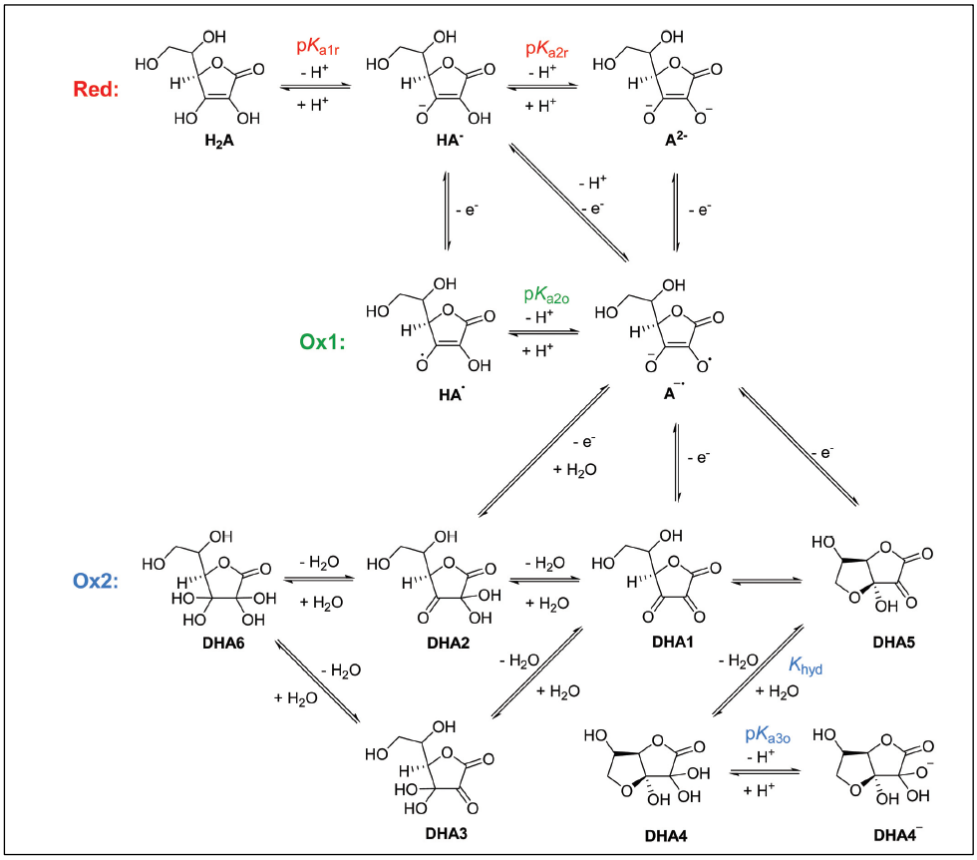


Figure 5: Top: pH-Dependent states of ionisation for vitamin C that are all still in their reduced state. Middle: First level of oxidation states for vitamin C. Bottom: second level of oxidation states for vitamin C. Source: Tu et al 2017.

It is also very important to note that once vitamin C has been oxidised it can be regenerated by multiple antioxidant and enzymatic processes in the body, which are largely associated with glutathione (a short, water-soluble peptide based on glutamate, cysteine, and glycine) and reductase enzymes which are in turn largely dependent on NAD^+/NADH and FAD^+/FADH metabolism (which are in turn dependent on nicotinamide/vitB3 and riboflavin/vitB2). Figure 6 illustrates how different nutrients within the system can be regenerated from their oxidised states back to their reduced states. The central basis for understanding here is to recognise that the cells have aqueous (water soluble) and membrane (fat/lipid soluble) environments that are biochemically different but are in close proximity. Therefore, as membranes become oxidised then nutrients including vitamin E may sacrifice themselves to protect the membranes for the degenerative effects of lipid peroxidation, and aqueous antioxidants like vitamin C may then sacrifice themselves to regenerate vitamin E. Vitamin C can be regenerated by either glutathione or by reductase enzymes that regenerate vitamin C directly back to its non-oxidised form. It is also worth noting that not all cells in the body have the same protein transporters for vitamin C, and in some important instances vitamin C is imported in its oxidised (DHA) form and then regenerated in the cell.

Nutrient Regeneration within a System

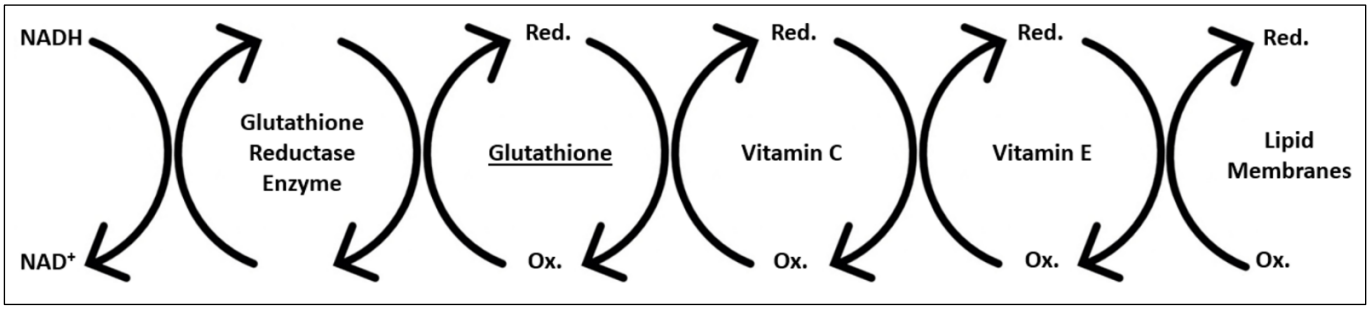


Figure 6: Basic schematic of the aqueous and membrane/lipid based antioxidant processes in the body and how nutrients can be regenerated from their oxidised (Ox.) state back into a non-oxidised or reduced state (Red.).

Because of these synergies between various vitamins, there is potential advantage in ensuring that vitamin C, vitamin E, vitamin B3 and vitamin B2 are present in appropriate ratios.

Biosynthesis of Vitamin C

L-Ascorbic acid (vitamin C) is synthesised in plants and in the livers of most vertebrates. Humans and most other primates lost the ability to synthesise vitamin C a long time ago on the evolutionary timescale due to a mutation in the GULO gene. In humans we call this genetic remnant GULOP, where the P stands for pseudogene as it is no longer functional. Some primates like lemurs in Madagascar still have a functional GULO gene and can still synthesise their own vitamin C and thus to not need to obtain it from their diets in the same way that humans and most other primates do.

Vitamin C as an Enzyme Co-Factor

Beyond vitamin C's role as a versatile electron donor and antioxidant, arguably the most important aspects of its functions in biochemical processes centre around its roles as a co-factor that are unique to its structure and earn it the title of a vitamin. Though briefly outlined above in the prevention of scurvy and a multitude of biochemical processes, the focus in the section will be upon collagen. There are many types of collagen and while most collagen might be type 1, it is important to have a balance of collagen types which are specific to different tissue types.

Figure 7b (on the next page) illustrates processes of synthesising collagen in the body. Procollagen proteins once synthesised by the ribosomes in the cell are subjected to post-translational modifications by hydroxylase enzymes that require vitamin C as a co-factor. Vitamin C also plays a role in other hydroxylation processes for example in the synthesis pathways for serotonin and melatonin, and thus is very important for prevention of mood and sleep disorders, however this will not be discussed here.

The hydroxylation processes in the development of collagen are necessary to allow the protein fibres to interact and knit into a collagen matrix. Clearly this is important from a structural integrity perspective for connective tissues, and whilst very noteworthy for the maintenance of healthy skin it cannot be understated that this is important for all tissues in the body.

Also, for collagen there are processes of glycosylation and glycation. These each refer to the attachment of carbohydrate/sugar molecules, however they differ in that the former is an enzymatically regulated post-translational modification and the latter is not and glycation processes are generally a negative outcome. These processes have been described for decades (e.g. Paul & Bailey 1996) but continue to be studied, in particular deepening our understanding about how increasing levels of advanced glycation end-products (AGEs) can be deleterious to health as we age. There has been some reporting that vitamin C may play active roles in inhibiting these glycation processes (e.g. Zafar et al 2012; Sadowska-Bartosz and Bartosz 2015; Van Putte et al 2016; Grzebyk and Piwowar 2016) but more research is required in this area. Figure 7a (on this page below) shows the difference between desired and undesired cross-links in collagen.

Collagen Cross-linking

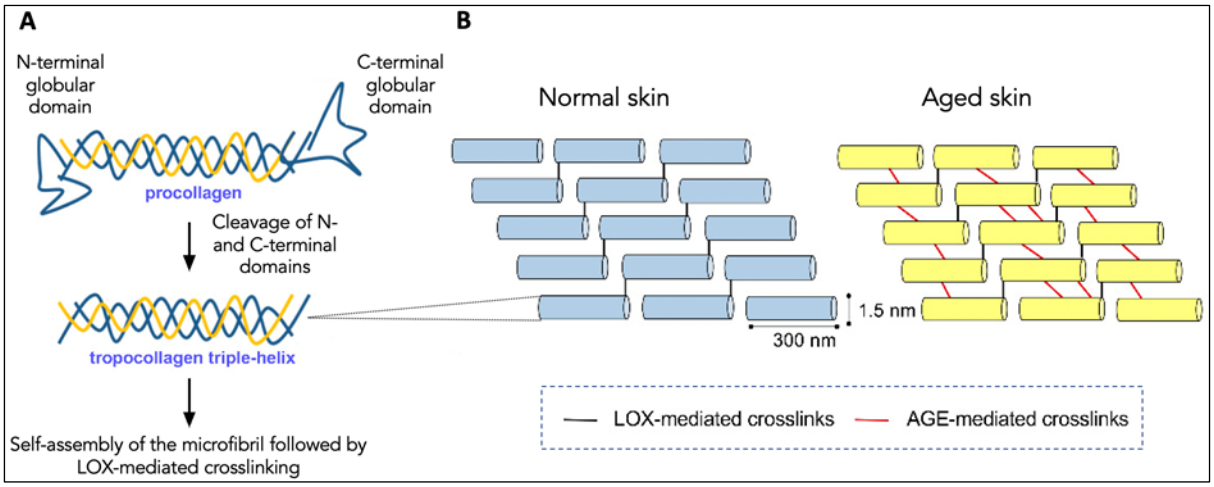


Figure 7a: Basic illustration of different forms of cross-links in collagen e.g. hydroxylation versus glycation. Source: McKay et al 2019.

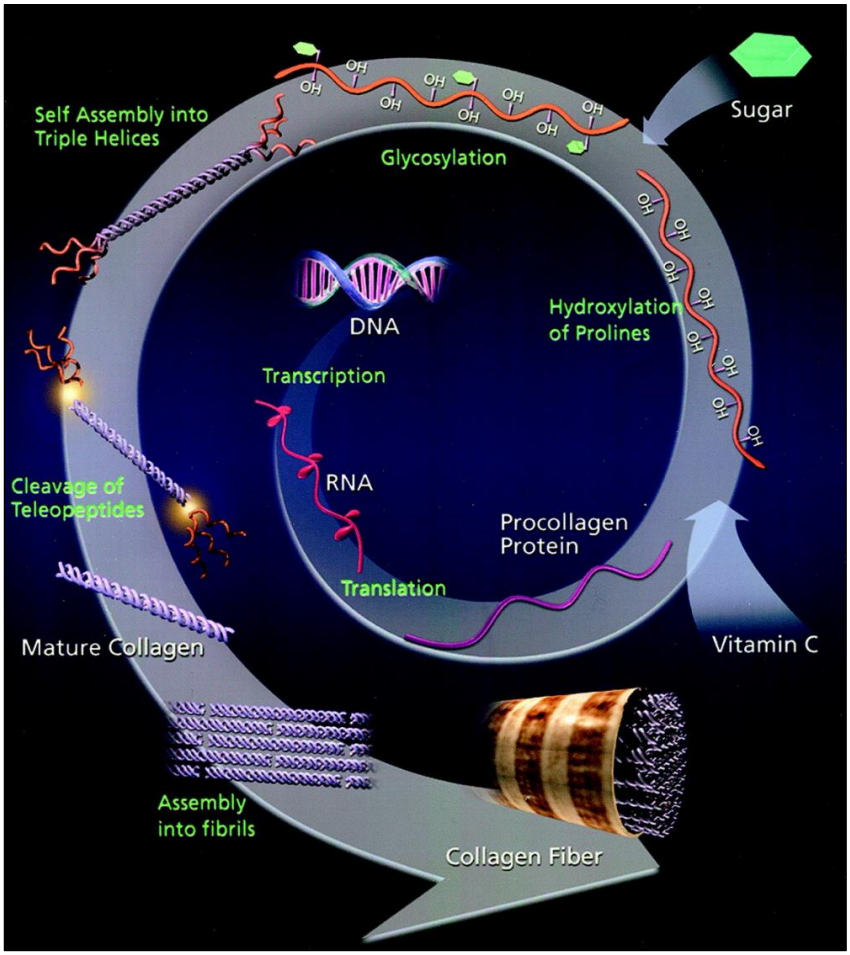


Figure 7b: Basic schematic for biosynthetic pathways for collagen production. Source: Libby & Aikawa 2002.

Vitamin C in Skincare (Oral versus Topical Application)

In the human body approximately 50% of vitamin C is found in the muscle (Low et al 2009), but this is because we have so much muscle mass, and the brain, eyes, and nervous system have much higher concentrations (potentially over 10-fold greater) of vitamin C relative to skeletal muscle. Heart muscle can have approximately up to three times the vitamin C level found in skeletal muscle. Levels of vitamin C measured in different tissues is illustrated in figure 8, and notably low levels get to the skin which provides some support of the use of vitamin C in topical applications, especially when the body is not achieving optimal levels from the diet, and in specific applications e.g. wound healing.

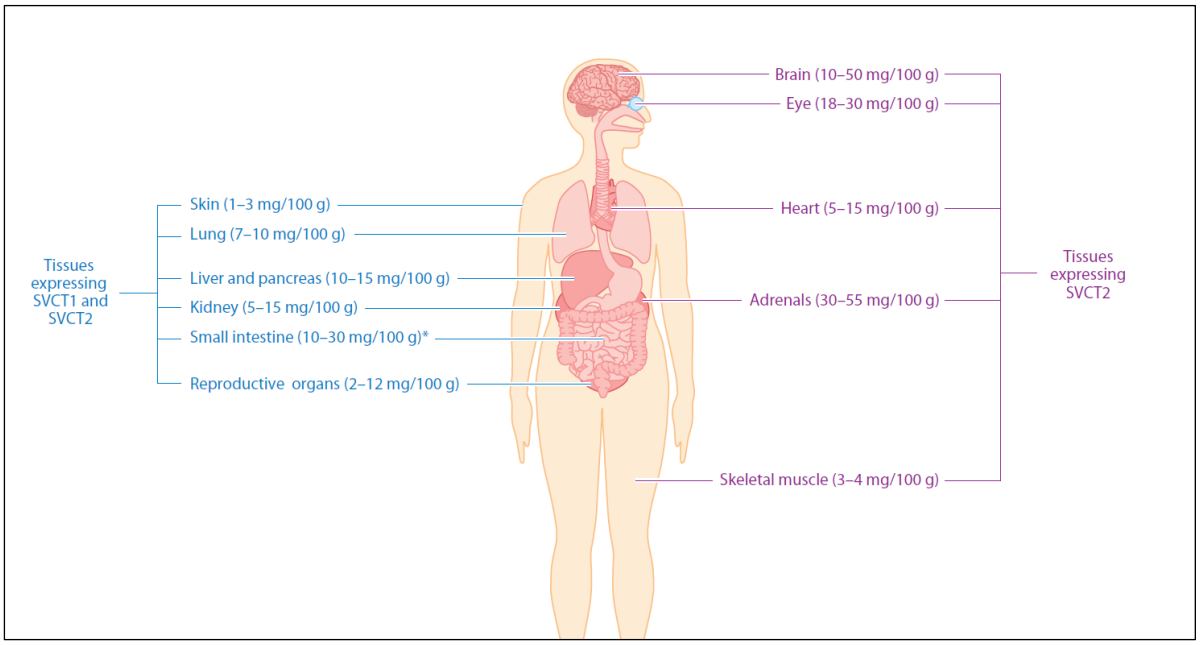


Figure 8: Outline of measured vitamin C levels in different human tissues. (Michels et al 2013).

If it is accepted that there may be benefit from applying vitamin C as a topical application to the skin, then the inevitable logic step is to consider the best formulations to be applied. The routes of oral and topical application are clearly very different for a number of reasons but most notably:

1. The oral route has specific protein transporters in the intestine (SVCTs and GLUTs) that will be directly exposed to the vitamin C to facilitate absorption, and the highly acidic environment of the stomach can enable hydrolysis of vitamin C conjugates.
2. The topical application route require the vitamin C to penetrate the stratum corneum layer of the skin to reach active cells of the epidermis and dermis that are equipped with SVCT transporter proteins to facilitate absorption of vitamin C from the external cellular environment into the active skin cells
3. For topical applications there may still be a value of having vitamin C in the formulation only to provide an antioxidant capacity for the formulation while in storage, but if the greater value of vitamin C for the patient/consumer is desired (which is generally the case) the seeking efficient absorption to active cells in the skin is clearly paramount

There have been a number of vitamin C conjugates/derivatives produced in the industry, largely for cosmeceutical purposes, but some of these seem to have been derived to focus on changing vitamin C from a highly water soluble molecule to one that is more fat soluble. Some of this has been driven by commercial interest rather than scientific evidence, and industrial producers have ignored some of the principles for effective skin penetration and absorption. There has been convincing, foundational research from the National Institutes of Health (Pinnell et al 2001, see figure 9) that low-pH (pH 3 to 4) applications of vitamin C in primarily ascorbic acid form (this reduces molecular charge) can have much greater absorption into the skin relative to other large molecules that are conjugations of ascorbic acid to other molecules e.g. ascorbyl palmitate is the conjugation of ascorbic acid and palmitic acid.

Using ascorbyl palmitate as an initial example for discussion the vitamin C has indeed become more lipohilic (fat soluble), however the size of the molecule is much larger which actually decreases its capacity to penetrate the skin. Also, there is a question around the purity of the produced ascorbyl palmitate versus palmitoyl ascorbate.

Ascorbyl palmitate – https://pubchem.ncbi.nlm.nih.gov/compound/54680660
Palmitoyl ascorbate – https://pubchem.ncbi.nlm.nih.gov/compound/9953598

With ascorbyl palmitate the palmitic acid has been conjugated to a hydroxyl group on the hydrocarbon tail, but with palmitoyl ascorbate the palmitic acid has been conjugated to a hydroxyl group on the ring structure which is one of the active sites responsible for the antioxidant properties. This significantly compromises the vitamin C's antioxidant capacity. For oral applications this is not such a concern because in the low pH stomach environment acid hydrolysis can occur which liberates the ascorbic acid, however for topical application the question of liberation remains. Thus, it is important to understand if the producers can verify that they have a pure product and not a blend. These molecules have exactly the same atomic formula, the only difference is the site of conjugation.

Sodium or magnesium ascorbyl phosphate, both make the ascorbate ion even more highly charged, so while the molecular size increase is only slight the increase in negative charge is quite significant, and the antioxidant capacity is compromised due to the conjugation site for the phosphate. Furthermore, with the example of tetra-hexyl-decyl-ascorbate (or ascorbyl tetra-isopalmitate) this has been taken to a more extreme level, creating a much larger molecule that has greatly reduced vitamin C function, simply to make the molecule as fat soluble as possible but now it is roughly 85% fat and 15% a compromised version of vitamin C. These things are perplexing to a molecular bioscientist and there is a paucity of evidence to support this product in any way. Despite this it appears to have become quite popular in the industry.

Dermal Absorption of Different Forms of vitamin C

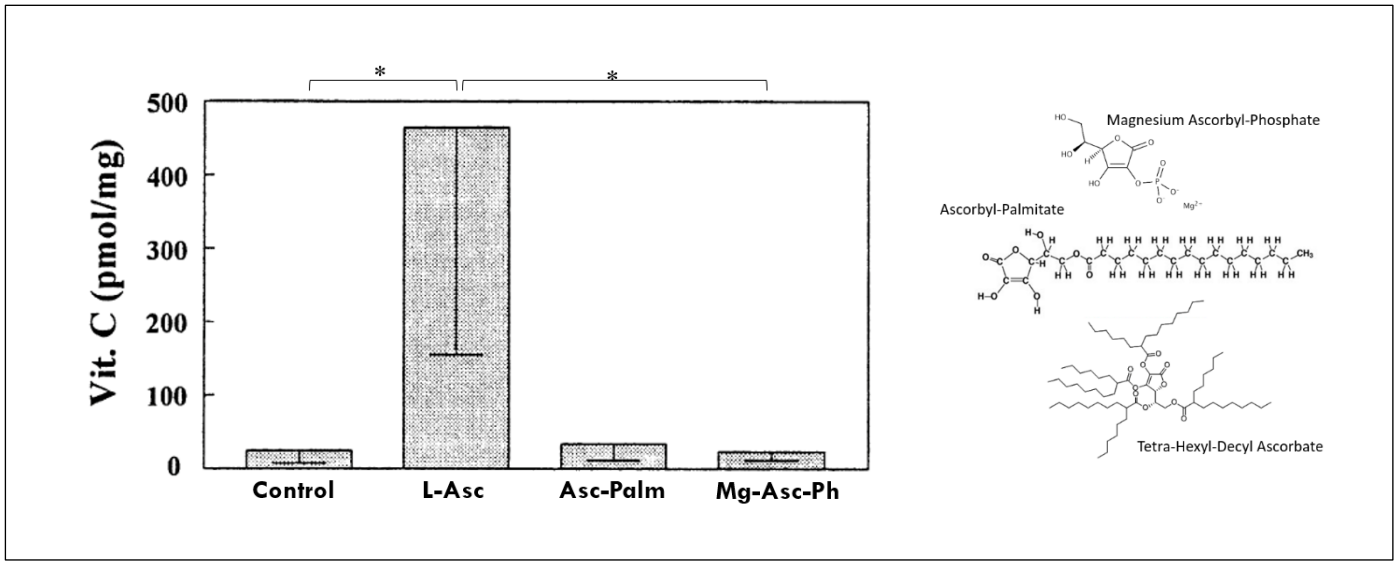


Figure 9: Vitamin C derivatives and disparity in skin absorption levels. Source: Pinnell *et al* 2013.

Evidence also shows that over 3 consecutive days that topical applications of ascorbic acid forms of vitamin C to the skin may increase levels to a saturation point, and that cessation of application may lead to a similar period of 3 to 4 days before levels return to baseline. Thus in the same way that regular oral application (1 to 3 doses per day, whether from diet or supplement) may increase blood levels above baseline line, regular topical application (once per day) may also increase levels of vitamin C above baseline in the skin. There is no significant evidence that topical application will actually influence blood levels and thus other non-proximal tissues.

While natural and synthetic vitamin C are exactly the same molecule with exactly the same properties, the process of manufacturing remains a significant consideration for formulation. Synthetic vitamin C is only as pure as the manufacturing process is verifiably clean and free from contaminants. Generally when vitamin C is extracted from highly valuable natural sources then a suite of other natural biochemicals may be extracted also.

NATIVE EXTRACTS Harnessing Natural itamin C

Native Extracts as performed qualitative and quantitative third-pasting testing on natural vitamin C extracts for years and demonstrated that the harnessing of the full suite of supporting biochemistry positively affects the stability of aqueous vitamin C solutions over a period of 24+ months.

Furthermore, NE has advanced their work on harnessing natural vitamin C from the Kakadu Plum in their essenXces methodologies achieving vitamin C levels as high as 16% (approx.16,000mg/100g) and in addition have the natural entourage of supporting biochemistry that is co-extracted from the valuable plant sources like Kakadu Plum.

Average Degradation of Ascorbic acid in Kakadu Plum Extract Concentrates (%) over 24 Months

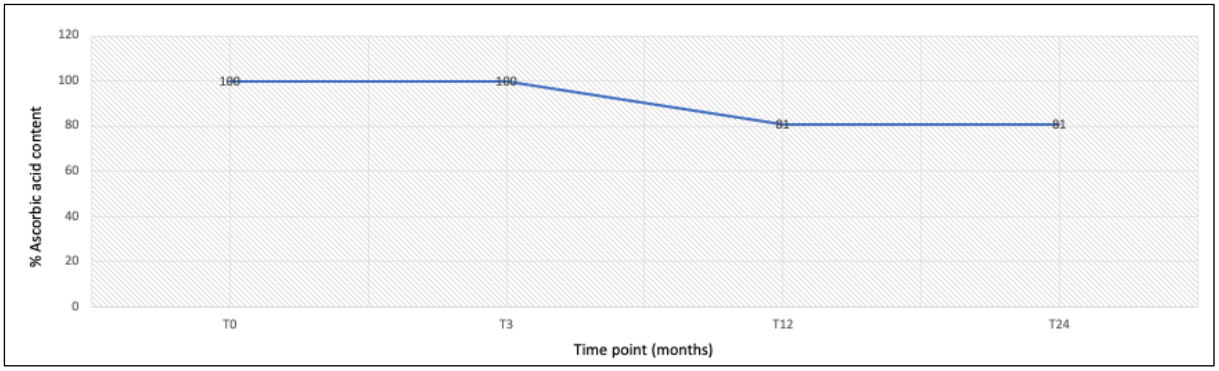
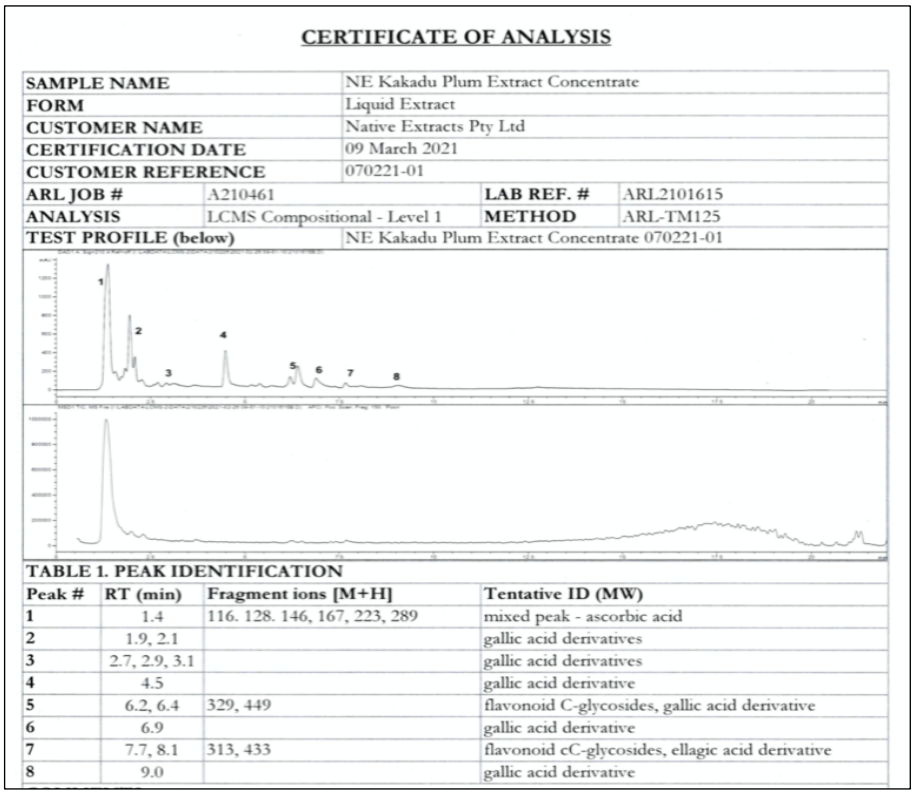


Figure 10: Vitamin C stability over time as determined by third-party testing. Average of multiple test runs.



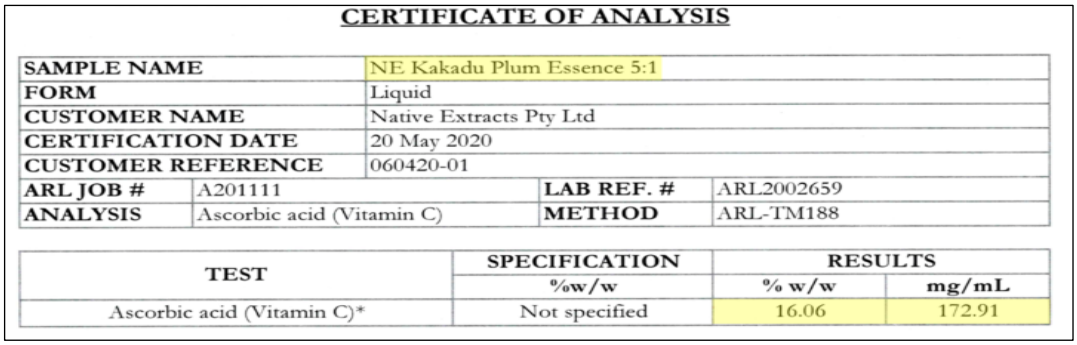
CERTIFICATE OF ANALYSIS

SAMPLE NAME	NE Kakadu Plum Extract Concentrate		
FORM	Liquid Extract		
CUSTOMER NAME	Native Extracts Pty Ltd		
CERTIFICATION DATE	09 March 2021		
CUSTOMER REFERENCE	070221-01		
ARL JOB #	A210461	LAB REF. #	ARL2101615
ANALYSIS	LCMS Compositional - Level 1	METHOD	ARL-TM125
TEST PROFILE (below)	NE Kakadu Plum Extract Concentrate 070221-01		

TABLE 1. PEAK IDENTIFICATION

Peak #	RT (min)	Fragment ions [M+H]	Tentative ID (MW)
1	1.4	116. 128. 146, 167, 223, 289	mixed peak - ascorbic acid
2	1.9, 2.1		gallic acid derivatives
3	2.7, 2.9, 3.1		gallic acid derivatives
4	4.5		gallic acid derivative
5	6.2, 6.4	329, 449	flavonoid C-glycosides, gallic acid derivative
6	6.9		gallic acid derivative
7	7.7, 8.1	313, 433	flavonoid cC-glycosides, ellagic acid derivative
8	9.0		gallic acid derivative

Figure 11: Example of Qualitative LCMS data, plant profile - NE Kakadu Plum Cellular Extract by third-party testing.



CERTIFICATE OF ANALYSIS

SAMPLE NAME	NE Kakadu Plum Essence 5:1		
FORM	Liquid		
CUSTOMER NAME	Native Extracts Pty Ltd		
CERTIFICATION DATE	20 May 2020		
CUSTOMER REFERENCE	060420-01		
ARL JOB #	A201111	**LAB REF. #**	ARL2002659
ANALYSIS	Ascorbic acid (Vitamin C)	**METHOD**	ARL-TM188

TEST	**SPECIFICATION**	**RESULTS**	
	%w/w	**% w/w**	**mg/mL**
Ascorbic acid (Vitamin C)*	Not specified	16.06	172.91

Figure 12: Vitamin C quantitative analysis Kakadu Plum Essence 5:1, determined by third-party testing.

Addressing Some Industry Myths

With proliferation of false information in the public domain, we address some circulating myths:

Vitamin C is not ascorbic acid - FALSE

- L-ascorbic acid and ascorbate ions are vitamin C

Vitamin C is extremely unstable and breaks down to in 15 seconds - FALSE

- Vitamin C can be stable when anhydrous and stored properly, and generally aqueous solutions break down in a timeframe of days to weeks, depending on formulation

Ascorbic acid is the used-up form of Vit C – FALSE

- Ascorbic acid and ascorbate ions are active forms of vitamin C

Ascorbic acid lacks any antioxidant activity and is so acidic it cases chemical burns – FALSE

- While there may be some issues with sensitive skin types, formulations of ascorbic acid at pH 4 are close to optimal skin pH

Ascorbate is the stable form of Vit C and is pH neutral – lacks any antioxidant activity – FALSE

- Both ascorbic acid and ascorbate ions are active

Ascorbyl palmitate has no antioxidant activity – FALSE

- Ascorbyl palmitate may have reduced antioxidant capacity relative to vitamin C but it is not zero

References

- National Institutes of Health, Office of Dietary Supplements (USA) https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/
- National Institutes of Health, National Library of Medicine, National Center for Biotechnology Information, PubChem https://pubchem.ncbi.nlm.nih.gov/compound/54670067
- Cochrane Collaboration (UK) https://www.cochrane.org/
- Oregon State University, Linus Pauling Institute, Micronutrient Information Center (USA) https://lpi.oregonstate.edu/mic/vitamins/vitamin-c

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About the Authors

Dr Evan Stephens, Senior Biotechnologist & Nutrition Specialist

Dr Evan Stephens PhD, B.Biotech, B.Sc(Hons) leads the research team at NATIVE EXTRACTS, driving advances in our Nutraceutical Division. Dr Stephens brings over 25 years' experience in sustainable agriculture, plant and nutrition science, 15 years in biotechnology research and technology commercialisation – with over 20 scientific publications, over 4,000 citations of his scientific work, and 4 fellowships (including senior research fellow at the University of Queensland's School of Agriculture & Food Sciences).

Evan shares NE's passion for discovery and scientific rigour to deliver nutrients from plants to improve human health and wellbeing. Dr Stephens is currently leading the team on numerous exciting new projects targeting Beauty, Wellness and Therapeutic applications: species-specific commercialisation projects, developing scientific papers for publication and peer review, and presenting industry seminars, such as our breakthrough in harnessing natural Vitamin C (biological ascorbic acid.

Lisa Carroll, Director, Owner & Innovator NATIVE EXTRACTS Pty Ltd
Lisa Carroll was instrumental in the inception of Native Extracts' (2012), it's direction, growth and communication of new discoveries and profiles of Australian natives, and connection to Indigenous and non-indigenous growers. Native Extracts is well known in the industry for their contributions in harnessing natural vit C (public release 2013) and more recent advances in concentrations, building new libraries of plant profiles of Australian native species and traditional botanicals. Dedicated to evolving the botanical extract to transparent, traceable natural ingredients delivering a "True to Nature" plant profile (water-soluble phyto-compounds, their derivatives etc), suspended in an entourage of supporting biochemistry, ready to formulate. She has been an influence behind the A(ustralian) Beauty trend, from ingredient through to consulting on brand development.

Lisa has built a highly experienced technical team with expertise across multiple extraction processes, new technology, R&D, and analytical plant chemistry from acclaimed universities, with an emphasis on conscious manufacturing practices. She continues to launch world first ingredients, build strategic partnerships to commercialise ingredients under licence, expand libraries of botanical ingredients and delivery formats. She is passionate about supporting the growth of an inclusive Australian native primary industry, and "powered-by-nature" solutions for Cosmetics, Pharmaceutical, Nutraceuticals, Beverage sectors.