Superfood

In search of the fountain of youth: Compounds that keep us young and let us live healthier lifes

By Guido Deussing

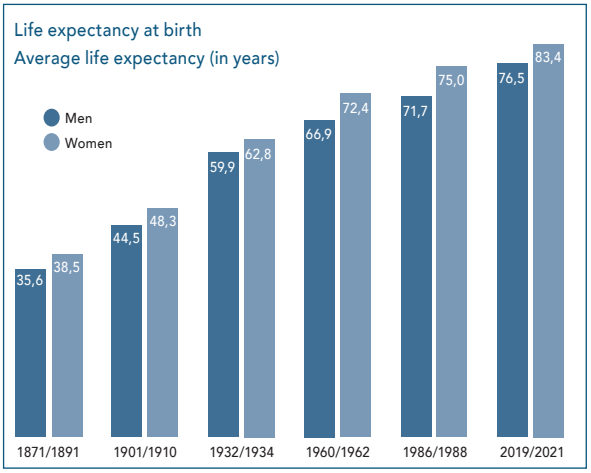
The biogenic polyamine spermidine has received a lot of attention since it was scientifically shown to slow down the aging process when taken in adequate quantities. Determining the spermidine levels in foodstuffs requires the right analysis strategy given that many have a highly complex matrix. Scientists at the Westphalian University in Recklinghausen, Germany have developed an efficient, fully automated method based on HPLC-TOF-MS to determine spermidine in the "superfood" Apilarnil.

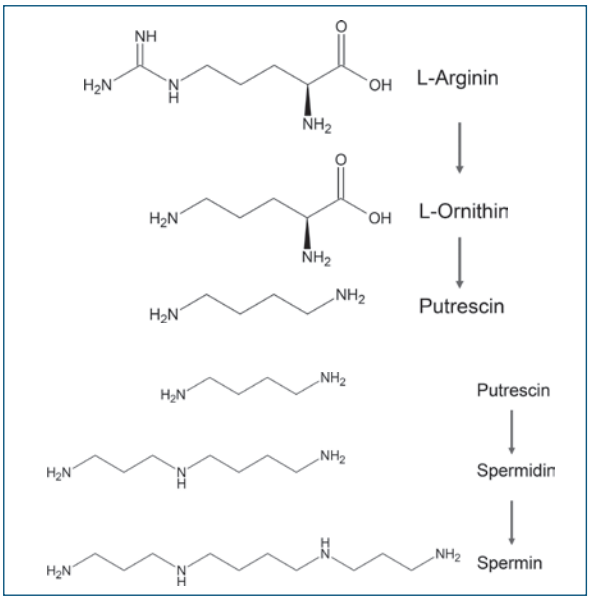
Wish and Reality

According to the Federal Statistical Office, life expectancy in Germany has almost doubled in the past 100 years: Girls born between 2020 and 2022 will live to an average of 83.2 years. On average, boys die five years earlier at 78.3 years old. But that doesn't for a minute stop us from wanting to live even longer and healthier lives. Life can be understood as a natural process, which after a point is characterized by progressive impairment of cell functions. Aging is part of life, but can it be slowed down?

Eat, Drink and be Merry

Regarding what determines our lifespan and quality of life, what we eat seems to be of great importance. There is widespread belief among nutritional experts that the Mediterranean diet, for example, has a positive effect on our health and can prolong life [1]. The diet includes large helpings of fruits and vegetables, as well as legumes, wholegrain products, and olive oil; add fish, milk and wine in moderation and meat in small quantities. Anti-agers and longevity seekers, among many others, want to know what makes this diet so valuable. It has been scientifically proven that the intake of certain foods has a positive effect on a person's lifespan.





Breaking Bad Habits

According to a Norwegian study, it is worth breaking bad eating habits even in old age, for example, avoiding fast food, eating less meat, and instead eating more legumes and whole grain products. After finishing their study [2], Fadnes et al. concluded that a 20-year-old can gain more than ten years of life with the right diet, and an 80-year-old more than three years of added life. At number one on the list of valuable foods are legumes such as lentils, beans, and peas. According to Fadnes et al., whole grain products and nuts are just as healthy as legumes. Fruit, vegetables, and fish also have a positive influence on a person's potential lifespan. Red meat and processed products such as sausage and ham have a negative impact, while eggs, poultry, processed cereals, and sugary drinks don't have much impact. Foods with antioxidant and anti-inflammatory properties counteract cell damage and age-related ailments such as cardiovascular disease, cancer, diabetes, and neurodegenerative diseases, such as Alzheimer's. Given that not everyone is able to eat the optimal amount of healthy food day in and day out, adding suitable dietary supplements is sensible. The European Food Safety Authority (EFSA) for one considers it helpful to compensate for nutritional deficiencies by maintaining an appropriate intake of relevant nutrients [3].

Cleaning and Recycling

One age-related change in the human body is that cellular cleansing processes deteriorate. The so-called autophagy is a type of recycling system that breaks down and removes unnecessary or damaged cell components, explains Evgeni Ponimaskin. This molecular clean-up mechanism keeps cells fit and protects against many diseases. However, as we age, the autophagy process stalls, but can apparently be reactivated with the compound spermidine, as the researcher from the Institute of Neurophysiology at the Hannover Medical University (MHH) states [4].

Picture caption (left hand side): Spermidine is an endogenous, natural substance, a polyamine first discovered in human semen, which gave the substance its name. We now know that spermidine is found in all cells in the body and that certain intestinal bacteria produce it as shown here. However, the majority must be absorbed through food. The foodstuffs that are known to contain the largest amounts of spermidine are wheat germ, cheese, especially Cheddar, soy products and legumes, and most of all in Apilarnil.

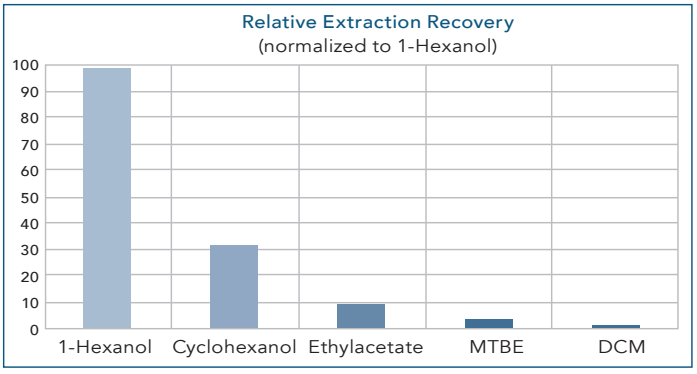


Figure X: When extracting spermidine from Apilarnil, 1-hexanol achieved the highest relative yield.

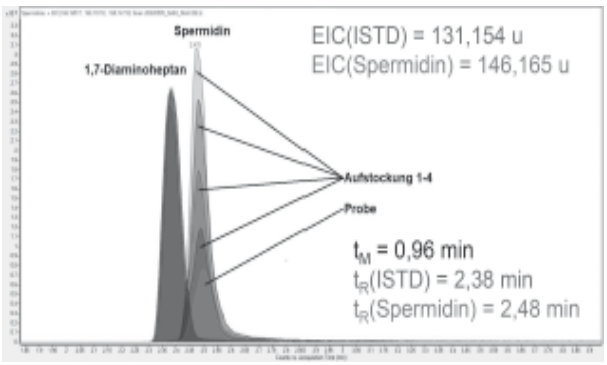
Heart and Mind

The anti-aging effect of spermidine has been examined in more detail by Ponimaskin and his research group in cooperation with the University of Graz based on animal experiments [3,4]. The researchers administered spermidine through drinking water to aged mice for six months and compared the results after the feeding period with those of untreated animals of the same age. As Ponimaskin and his team report, clear anti-aging effects were observed in the treated animals. The spermidine intake ensured that the animals developed less kidney and liver damage and improved the performance-enhancing glucose supply in the brain. Age-related hair loss was also significantly lower than in the control group. The animals supplied with spermidine hardly showed any bald spots on their backs as normally seen in older mice.

The heart-protecting effect of spermidine was particularly interesting to the scientists. In their studies, they report finding that the cardioprotective effect

is associated with reduced telomere shortening in heart muscle tissue. Telomeres protect the ends of chromosomes, carriers of genetic information stored in our body cells, from degeneration. Each time a cell divides, the telomere ends are shortened slightly. In cells that are no longer dividing - such as heart muscle cells - the telomeres are further shortened until a critical length is reached and so-called programmed cell death occurs. To their delight, the researchers found that telomeres in the spermidine-supplemented mice were similar in length to those in young animals. Since the aging processes in the cells of mice are similar to those in human cells, taking spermidine, for example as a dietary supplement, could also protect against age-related problems in humans, while strengthening cognitive processes and memory. To check whether the results from the animal model can be transferred to humans, researchers from Austria and Germany [6,7] used data from the Bruneck study. A group of 829 participants with normal cognitive performance in 1995 was selected. Those of the subjects who had developed cognitive impairments over the following five years of observation were identified using the neuropsychological test battery CERAD (Consortium to Establish a Registry for Alzheimer's Disease). The domains of memory, executive functions (planning) and language skills in the brain were examined. Spermidine intake from food was then determined. The results show that study participants who ingested more food with high spermidine levels, such as wholegrain products and legumes, in 1995 showed significantly less cognitive loss over the following five years.

Nutritional supplements containing alpha-ketoglutarate, hyaluronic acid, NAD+ boosters, vitamin D3 and vitamins B7, B9 and B12 are advertised as "longevity products". As part of his rejuvenation treatment, the billionaire Bryan Johnson takes 13.5 mg of spermidine in his "The Green Giant" smoothie every morning, apparently with some success in reversing the aging process. The "Blueprint" rejuvenation project can be followed on his Webpage [5]. As recent studies suggest [4], spermidine does appear to influence the aging process.



Experiments and Evidence

Various studies have produced reliable evidence of positive effects of spermidine on human health. Examples are: Improved autophagy, reduced risk of cancer, improved cardiac health, effective protection against Alzheimer's and Parkinson's, reduced risk of developing type 2 diabetes, as well as a reduction in high blood pressure, as Prof. Dr. Ingo Tausendfreund from the Westphalian University in Rechlinghausen, Germany explains. The Bruneck study by research teams from Graz and Innsbruck with 829 test subjects even showed that people whose diet was rich in spermidine had a significantly lower risk of dying over the 20-year observation period.

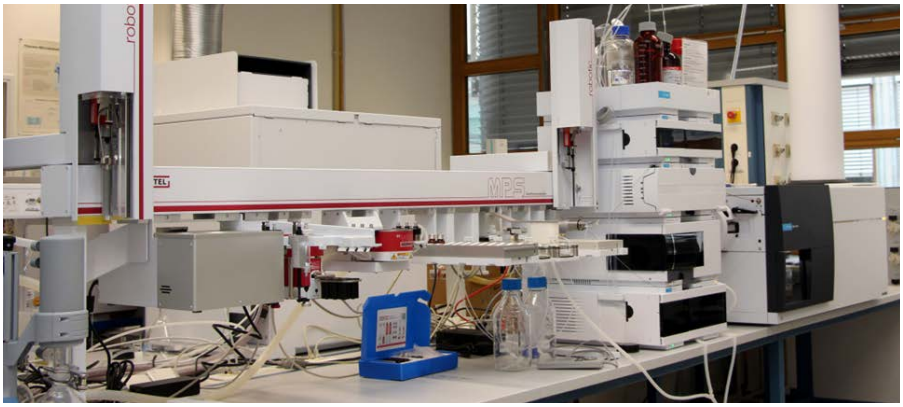
Valuable and Content Rich

Foodstuffs that are rich in spermidine have been categorized as "superfoods", to be used for life-prolonging and rejuvenation treatment plans. The following foodstuffs are valuable sources of spermidine (Spermidine content listed per 100 g): avocado (1.0 mg), potato (1.2-1.7 mg), hazelnuts (2.1 mg), mango (3,0 mg), cauliflower and broccoli (2.6-3.7 mg), pear (5.2 mg), peas (6.5 mg), shiitake mushrooms (8.9 mg), aged cheddar (19.9 mg) and wheat germ (24.0 mg). Those more interested in business aspects can take note that a market analysis for "superfoods" has projected growth from 165 billion US dollars in 2023 to 269 billion US dollars in 2028 [8].

Speaking of superfoods: Beekeepers (apiarists) in Germany offer ostensibly performance enhancing bee preparations under the name Apilarnil an extract of freeze-dried and powdered drone larvae, which contains spermidine, beta-carotene, choline, vitamins A, E, B1, B2, and B6, as well as numerous minerals (calcium, phosphorus, sodium, potassium, and magnesium) along with trace elements. The discovery that drone larvae extract can be a valuable nutritional supplement came from a chance observation: Ducklings fed drone honeycombs grew faster than a reference group.

Bee Larvae Prepared

A company specializing in the marketing of apiary products approached to determine the spermidine content in freeze dried drone larvae, a challenging task due to the complex biological matrix. A completely automated approach was required to process large numbers of samples. LC-MS/MS was identified as the method of choice; diode array detection (DAD) would require considerable added sample prepara-



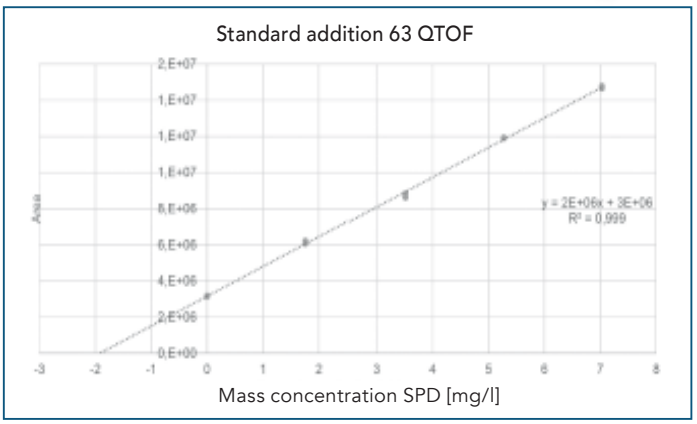
tion effort and derivatization steps with added uncertainty. The planned analysis steps were: 1. Selective extraction of the biogenic amine spermidine from the matrix; 2. Chromatographic separation; 3. Determination with a quadrupole time-of-flight (QTOF)-MS.

In initial work, 1-hexanol was found to produce the highest relative extraction yield as shown in the Figure on page 21. 1,7-diaminoheptane was added as internal standard in a standard addition process. Using the GERSTEL MultiPurpose Sampler (MPS) robotic, the researchers performed preliminary tests, optimizing the process regarding the sample amount, internal standards used, calibration strategy, and various method parameters (volumes, mixing times, centrifugation, wash cycles, needle penetration depths, etc. (For details on the method, please contact info@gerstel.de).

The MPS robotic, equipped with quickMIX and centrifuge enabled complete automation of all steps in the workflow from sample preparation to sample introduction. The analysis was performed using an Agilent 1260 UHPLC with a Restek Force Fluorophenyl column (3.0 µm, 150 x 2.1 mm) using a solvent gradient. Mass-selective detection was performed using an Agilent QTOF 6546 (Dual AJS ESI source) in positive mode (TOF-only). The analysis can also be performed using an LC-MS/MS system (precursor ion m/z 146.00, daughter ion m/z 72.00).

Results and Outlook

A series of apilarnil samples were analyzed with spermidine levels ranging from 310 to 459 mg/kg, averaging 386 mg/kg. The relative standard deviation of the determination was 1.5 percent with linearity (R^2) greater than or equal to 0.999. The analysis method was successfully developed and automated based on the MPS robotic-LC-QTOF system. The following are amounts of spermidine per 100 g: Peas: 6.5 mg; aged cheddar: 19.9 mg; wheat germ: 24.0 mg. Apilarnil weighed in at a whopping 38.6 mg per 100 g. Whether it can extend our life expectancy – or keep us (feeling) young remains to be seen.



Standard Addition calibration curve for spermidine in Apilarnil demonstrating excellent linearity

The automated method can be used to determine spermidine levels in other foodstuffs if the buzz surrounding spermidine continues. The hope is that finding other sources of this promising nutrient will ultimately lead to more and extended well-being for the wider population.

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