BIOSENSOR
FOR
POLYAMINES

MSc dissertation report by

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STATEMENT

I hereby declare that the mater presented in this school for dissertation entitled biosensor of polyamines is based on the result of investigations carried out by me in the School of Chemical Sciences, Goa University under the supervision of **Dr. Kanchanmala Deshpande** and the same has not been submitted elsewhere for the award of degree or diploma.

Rahul C Desai
PR.20P0460003

CERTIFICATE

This is to certify that the dissertation entitled biosensor for polyamines, is a Bonafide work carried out by **Mr. Rahul Desai** under my supervision in partial fulfilment of the requirement for the reward of the degree of Masters of Science in Chemistry at School of Chemical Science, Goa University.

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ABSTRACT

Polyamines (PA's) are low molecular weight aliphatic nitrogenous bases containing two or more amino groups. They are produced by organisms during metabolism and present in all cells. They are considered as plant bio-stimulant. The development of polyamines by molecular biotechnology technique, if there is increase in PA's, whether applied exogenously or produced endogenously via genetic engineering, can affect positively towards plant growth and stress tolerance. Polyamines are small aliphatc amines that are found in both prokaryotic and eukaryotic organisms. These growth regulators have been implicated in abiotic and biotic stresses as well as plant development and morphogenesis. Polyamines have also been linked to fruit ripening and in the regulation of fruit quality related traits. Polyamines which are also regarded as indicator of food freshness or spoilage and for evaluating microbial action while food processing. Polyamines have various adverse effects on human health they are widely found in varying method for concentration in different food products. Polyamines levels in humans have both positive as well as negative effects. Biosensor have emerged as an efficient tool for rapid and accurate analysis. In this article work reviews about existing technologies to analyze polyamine and reported biosensor as excellent tool for polyamine analysis among the existing techniques. (1,2,3,4,5)

Approach for literature search

The keywords polyamines, food, food analysis, fruits, plants, diet, biosensors, and the particular names for the three polyamines chosen for inclusion in the database: putrescine, spermine, and spermidine were used to search databases extensively. PubMed, Web of Science, and Scholar were used to conduct this search, which included both Medline and CAPLUS databases. Any published laboratory data for polyamines content in foods was found using both Medical Subject Headings (MeSH) and text word searching. The papers were chosen from those published between 2010 and 2022.

History of polyamines

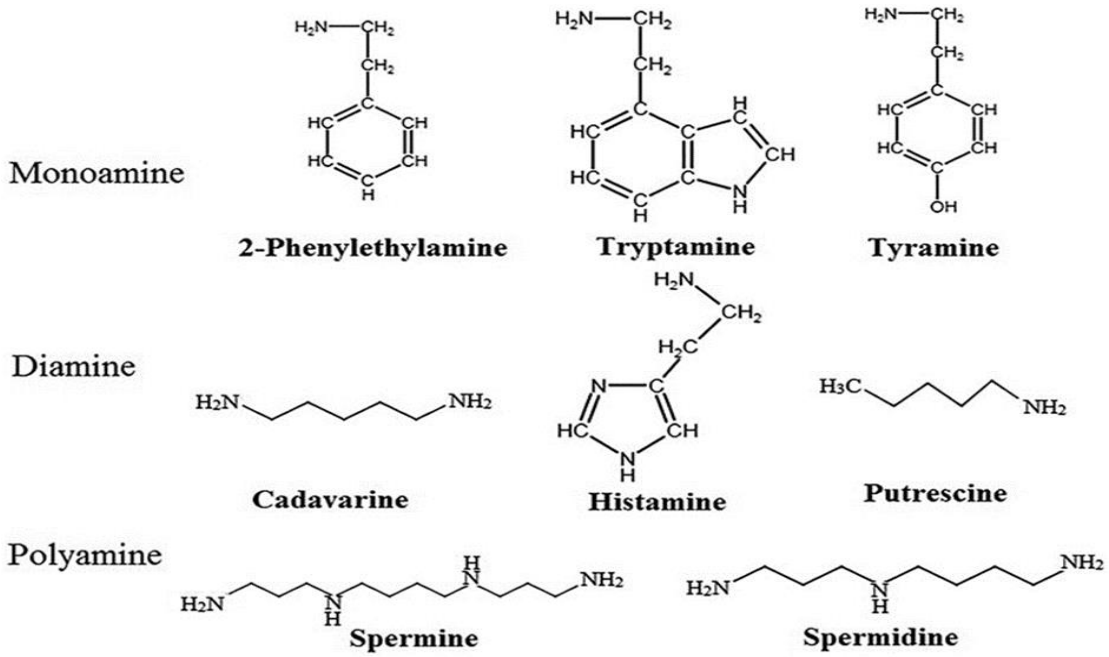
History of Polyamines and development was started back in 1674 when Antonie Van Leeuwenhoek identified crystalline compounds in human sperm. Rosenheim and colleagues did not discover the specific composition of polyamines and synthesized them in their laboratory until 1924. Many contributions to the field of polyamines were

made after Leeuwenhoek's, for example, they proposed theories on semen crystals. The crystals could be calcium phosphate, according to Vauquelin's prediction, but they could also be proteins, according to Boettcher. Ladenburg and Abel described organic crystals in 1888 and gave them the Latin name 'spermine'. The chemical makeup of spermine was discovered around 250 years after Leeuwenhoek's report. (6,7)

Introduction

Polyamines are small positively charged molecules that are found in virtually all living cells. They are essential for living in eukaryotes and play critical roles in multiple cellular purpose. Maintenance of the appropriate polyamine level is necessary to allow these functions, and excess polyamine levels can lead to toxicity. In living organisms, they may exist in free polyamines form or covalently conjugated form or non-covalently conjugated form this covalently conjugated polyamines can be further divided into perchloric acid-soluble covalently conjugated polyamines (PSCC-PA's) and perchloric acid-insoluble covalently conjugated polyamine (PISCC-PA's). In higher plants, PA's are mainly present on their free forms. Putrescine (Put, spermidine (spd), and spermine (spm) are main PA's in plants, and they are involved in the regulation of diverse physiological processes. such as flower development, embryogenesis, organogenesis, senescence, and fruit maturation and development. They also involved in responses to biotic and abiotic stresses. Free polyamines covalently bind to biomacromolecules, such as proteins, nucleic acid, uronic acid, or lignin by ionic and hydrogen bonds to form bound PA's also known as prichloric acid-insoluble covalently conjugated polyamine (PISCC-PA's). In physiological PH range, free polyamines (F-PA's) are fully protonated and positively charged, and can electrostatically combine with negatively charged biomacromolecules, such as protein, membrane phospholipid, nucleic acid in the organisms. Dietary polyamines have an important role in human health, mainly in intestinal maturation and in the differentiation and development of immune system. The antioxidant and anti-inflammatory consequence of polyamine can also play an important role in the interference of chronic diseases such as cardiovascular diseases. In addition to endogenous synthesis, food is a crucial source of polyamines. Although there are no recommendations for polyamine day-to-day intake. *de* novo synthesis of polyamines tends to decrease with age, which is why their dietary sources acquire a greater importance in an aging population. Polyamines can be found in all types of foods in a wide range of concentrations.

Spermidine and spermine are unnaturally present in food whereas Putrescine could also have a microbial source. The main polyamine in plant-based products is spermidine, whereas spermine content is generally higher in animal-derived foods. In 1678, Antoni van Leeuwenhoeck discovered the presence of crystals in human semen, which 200 years late (1888) were named spermine by A. Landenburg and J. Abel. The chemical composition of spermine and spermidine was determined in 1924 (1). The polyamines spermidine (N-(3-aminopropyl)-1,4-butane diamine), spermine (N, N-bis(3-aminopropyl)-1,4-butane diamine), and Putrescine (1,4-butane diamine) have low molecular weight. polyamines are stable compounds, capable of resisting acidic and alkaline condition and they can constitute hydrogen bonds with hydroxyl solvents such as water and alcohol. (8,9,10,11,12)



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(https://doi.org/10.1080/07388551.2019.1680600)

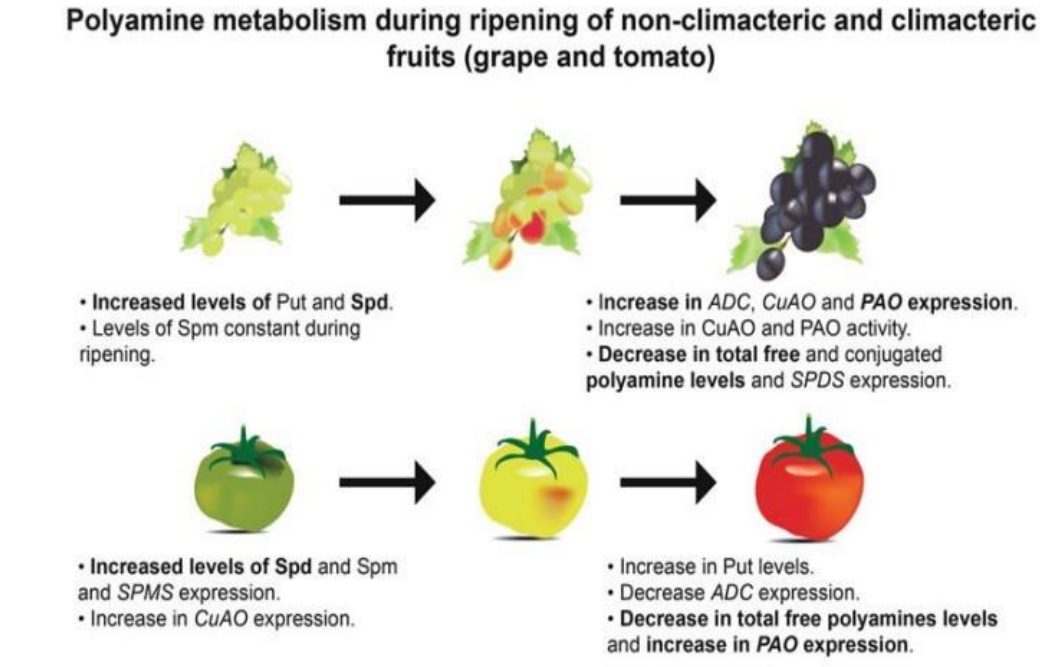
Polyamines present in plants

Polyamines are present in eukaryotic and prokaryotic cells and are found in plant RNA viruses and plant tumors. They have potency biological activity. They are numerous forms of PA's. In higher plants PA's are preponderantly present in their free form. The most ordinary PA's in higher plants are Put, Spd, Spm, thermospermine (Tspm) and cadaverine (cad). other polyamines are found only in certain or under special conditions. Polyamines show tissue and organ specific arrangement patterns in plants. For example,

most abundant PA in laves was found to be Put, and its level were higher than those of Spd and Spm, whereas Spd was found to be most ample PA in other organs. Different types of PA's also show different localization patterns within cells. In carrot cells, put was found to pile up in the cytoplasm, and Spm in cell wall. The distribution patterns of PA's may be connected to their unique functions. In general, more vigorous plant growth and metabolism is related with greater PA biosynthesis and higher PA contents. (13,14,15,16)

Polyamines in fruits

A suitable fruit development, followed by a correct growth, guarantee fruits with an excellent quality for the consumers. Ripening is the process in which fruit organic chemistry and physiology are altered including alteration in color, texture, aroma, flavor, and nutritional characteristics. Polyamines catabolism is progressive in several development processes such as fruit ripening and leave senescing. In fact, when exogenous PA's are provided to fruits improvements are detected in quality features such as fruit set, fruit size, post-harvest decay and decreased fungal infection. Agudelo-Romero and coworkers showed that polyamine catabolism may play a role in grape ripening. Previously, a gain in GABA was detected during grape ripening suggesting that the oxidation of polyamines produced a decreased in the polyamine titers. (16,17,18)



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(https://link.springer.com/protocol/10.1007/978-1-4939-7398-9_360)

Fruit ripening is interdependent on a highly coordinated network of endogenous and exogenous signals affect hormones among others. Fleshy fruits are grouped into climacteric and non-climacteric, depending on the presence or absence of the climacteric gain in the respiration and ethylene production during ripening. Indeed, in climacteric fruits (i.e. tomato, banana, apple, pears, mangoes, papaya, and avocado), ethylene comprise a major cue that controls most ripening features. (16,17,18)

Polyamines in food

Polyamines are found in foods of animal and plant origin, either free or conjugated. Conjugated polyamines are found in plant foods, primarily in connection with phenolic compounds. In foods, spermidine and spermine occur primarily naturally from raw plant and animal tissues, but putrescine is also formed by the activity of fermentable or contaminating microorganisms. It has also been explained that spermidine and spermine may be of partial bacterial origin, especially in fermented products. Therefore, treatment and storage conditions can affect the total polyamine content. (19,20,21,22)

Polyamines in Breast Milk and Infant Formula

Initial dietary exposure to polyamines is via breast milk. **Table 1** shows the levels of polyamines in breast milk and infant formula reported in the literature. All results are expressed in nmol / mL for ease of comparison. All studies reviewed agree that the levels and profiles of these compounds may vary due to factors such as genetics, lactation, age, maternal nutritional status, and food intake. The most important polyamines in breast milk are spermidine and spermine, their levels vary considerably, with coefficients of variation exceeding 68% and 53%, respectively. Spermine levels are generally high, except for two studies by the same author who reported high levels of spermidine. Breast milk analyzed in different studies corresponds to different stages of breastfeeding and may contribute to the high variability observed. In this sense, some authors state that polyamine content tends to decrease during lactation. In addition, two studies found higher levels of polyamines in breast milk in preterm infants compared to full-term infants. As a preliminary note, it should also be noted that milk from overweight mothers contained less polyamines than milk from mothers of normal weight. For infant formula, the variability between the results of various studies is even greater than for breast milk, with a coefficient of variation of 89% for putrescine, 116% for spermidine, and 160% for spermine. Despite this variability, it can be estimated that

the polyamine content and polyamine profile of infant formula are different from those found in breast milk.

Table no. 1 - Level of polyamine in breast milk.

Breast milk	**Putrescine**	**Spermidine**	**Spermine**
Full-term	0.896	3.849	3.440
A	0.615	3.512	4.490
Pre- term	0.058	0.462	0.302
B	1.655	6.151	1.677
First formula	3.880	2.265	0.363
	3.596	0.516	0.302

For representation, the leading polyamine in infant formula is putrescine, its usually higher than in breast milk, whereas spermidine and spermine levels tend to be lesser. Similarly, the few available information on polyamines in infant formulas for immature babies do not allow to observe variation with other types of formulas. The available information on polyamine content in breast milk and infant formula are rare and in some cases, out-of-date. More studies are needed to explain whether the variability observed both in breast milk and infant formula is due to the use of different analytical method or to other factors that have not been sufficiently examined. (22,23,24,25)

Food of Plant Origin

Polyamines are ubiquitous in plant-based foods, but their levels and distributions vary by food type **Table no. 2**. Spermidine, found in all plant-based foods, is generally the major polyamine. The food categories with the highest levels of spermidine and spermine are cereals, legumes and soy derivatives. Of particular note are wheat germ and soybeans with corresponding values of 2,437 and 1,425 nmol / g for spermidine and 722 nmol / g and 341 nmol / g for spermine. Mushrooms, peas, hazelnuts, pistachios, spinach, broccoli, cauliflower, and green beans also contain significant quantity of both polyamines. The lowest level is in the fruit category for example, spermidine levels reported for apples, pears, cherries, oranges, or mandarins are <21 nmol / g and <1.98 nmol / g for spermine. Like spermidine, putrescine is found in virtually all plant-derived foods, especially in fruits and vegetables, especially citrus fruits (1,554 nmol / g) and

peppers (794 nmol / g). Wheat germ (705 nmol / g) and soybean sprouts (507 nmol / g) also contain high levels of putrescine. Variations in the polyamine content of plant-derived products can be due to several factors, including their origin, growing conditions, harvest or storage. In this sense, various stress conditions in plants can affect polyamine content. For example, polyamine levels in plants can increase in response to high or low cultivation temperatures or stress from drought. Studies have shown that applying polyamines before and after cultivation compensates for the adverse effects of cold and drought and favors germination, plant growth, or survival. Another factor that can explain the high levels of putrescine in some vegetables, such as spinach and peas, is spoilage bacteria, primarily because of Enterobacteriaceae and Clostridium spp. existence. They produce putrescine from the amino acid precursor ornithine by amino acid decarboxylase activity. (25,26,27,28,29,30)

Table no. 2- Polyamines levels found in all plant-based foods.

Food categories	**Putrescine**	**Spermidine**	**Spermine**
Fruits Apple, avocado, banana	0-1,554	6.9-9.8	0-24
Vegetable Broccoli, cabbage, carrot.	5.7-794	69-398	0-54
Legumes and soybean Chickpeas, peas, soybean.	0-525	1-1,425	0-341
Nuts and oil seeds Almond, chestnuts, pistachios.	34-488	41-383	63-165
Cereals Rice, wheat, bread.	2.3-704	2.8-2,437	0-722

Polyamines in tea

Tea is a widely available product that is consumed in great quantities on a regular basis all over the world. Tea extract is made from Camellia sinesis leaves and buds combined with water. Every country uses different plant types, quantities, quality, and procedures. Green tea, after heat or stream treatment and rapid drying of young leaves and buds of the plant, and black tea, after weathering, destruction of plant tissues by various rolling, crushing, and/or tearing, and ultimately drying, are two well-known categories. Following these steps, the enzymes responsible for oxidation and breakdown are released, resulting in the formation of polyphenols and color compounds. (31,32)

Food of Animal Origin

The content of polyamines in animal-derived foods, such as plant-derived foods, is highly uncertain **Table no. 3.** Meat and its ingredients may contain high levels of spermine and spermine. In particular, samples of beef, pork, chicken, prosciutto and sausage showed no significant difference between fresh meat and derivatives, with the latter spermine value > 148 nmol / g. In fish and fish products, spermine and spermidine levels are generally lower than in meat products, but significantly higher than in lower levels of milk and eggs. Most cheeses have spermine and spermidine values of, <10 and 69 nmol / g, respectively, except for blue cheese (262 nmol / g), which has a very high content of spermidine. In fresh animal-derived products (meat, fish, milk, eggs), putrescine levels are generally lower than in plant-based foods. However, higher levels of putrescine are found in products that undergo fermentation processes that potentially involve aminogenic microorganisms. Widespread putrescine levels can also be explained by the decarboxylase activity of spoilage bacteria. Studies show that the hygiene of raw materials has a significant impact on the formation of putrescine and other amines in the production of various foods. For example, it has been reported that more amines accumulate in dry-fermented sausages when made from raw materials with low microbial quality. This factor can also contribute to increased levels of spoilage in long-aged cheese, where the use of raw milk is an accepted practice in manufacturing. In this sense, the heat treatment of milk is a useful tool not only to ensure the absence of pathogenic microorganisms, but also to avoid the formation of putrescine and other biogenic amines.

A) Amount of putrefactive microorganisms with amino acid decarboxylase capacity.
B) Presence of free amino acid precursors by delaying proteolysis during maturation.
C) Concentration of thermolabile pyridoxal phosphate, a necessary cofactor for the amino-acid decarboxylase enzyme. (33)

Table no. 3 - Polyamines levels in animal-derived foods.

Food categories	**Putrescine**	**Spermidine**	**Spermine**
Fresh meat Beef, lamb, chicken.	1.1-47	1-92	1-342
Cooked meat derivative Cooked ham, wiener sausage.	4.5-11	15-28	11-99
Cured and fermented meat Dry-cured ham, sausages	5-1771	8-62	11-177
Semi preserved and canned fish Canned tuna	0-487	0-167	0-111
Egg	3.1-10	1-4	0-1
Milk and dairy products Milk, yogurt	0-3	0.41-5	0-4

Effects of Culinary Treatment

Culinary treatment can potentially decrease the polyamine content in foods by two possible mechanisms: (a)transfer to the cooking water or

(b)due to the high temperatures reached in some types of cooking.

The few survey evaluating the consequence of culinary treatment on polyamines report uncertain results, depending on the type of cooking and the food studied. Polyamine contents after the cooking of certain vegetables (spinach, cauliflower, and potatoes) were significantly decreased by transfer to the cooking water, specially putrescine, as this is the most water-soluble polyamine. However, the similar cooking process did not induce losses in other types of food (peppers, peas, and asparagus). Another study found no significant differences in polyamine levels between raw and boiled vegetables (carrots, broccoli, cauliflower, and potatoes), although the low number of samples analyzed (two per food type) was a limiting factor. No significant loss of spermidine

and spermine was observed in meat that had been cooked with large amounts of water (boiled and boiled). For some cooking techniques with high temperatures, roasting, grilling, or fried foods have been described as resulting in a loss of up to 60% of spermidine and spermine in chicken. (34)

Polyamine Intake

The day-to-day intake of polyamines has been estimation for different European countries, Japan and the United States (Table 4). The mean polyamine intake in the European fully grown population was estimated as 354 μmol/day, with differences among the member states, being least in the United Kingdom and higher in the countries of the Mediterranean area, Italy and Spain. Subsequent studies carried out in Mediterranean countries, such as Spain and Turkey, have estimation of much lower intake values for their populations, which could be partly related to a decrease in the consumption of plant-derived foods due to the progressive abandonment of the traditional Mediterranean diet observed in the last 20 years. The polyamine intake estimates for the fully grown population of Japan and the United States lie between the European mean and the values corresponding to the Mediterranean area. (36)

Table no. 4 - The day-to-day intake of polyamines has been estimation.

Country	Total	Putrescine	Spermidine	Spermine
European union	352.6	211.9	87	54.1
United states	315	160	96	58
Turkey	343	222	71	49
Japan	200	90	74	36
Spain	249	159	54	35
Asian country	576	106	91	38

The differences between intake estimates can be attributed not only to the different dietary patterns of each population, but also to the age group studied, the methodology of data collection and/or to the variability in food polyamine content. For example, the food consumption data used to estimate polyamine intake was obtained from published national surveys (Japan and Spain), a frequency-of-consumption questionnaire (United States), a 7-day food record (Sweden) and a 24 h dietary recall (Turkey). In some studies, the data on polyamine content were obtained from analyses carried out specifically for the intake estimation studies, whereas others used data already published in the literature. All the studies agree that the polyamine contributing most to the total intake is putrescine, mainly from the consumption of fruits and vegetables, or in Japan also from cereals and soy sauce. Fruits, vegetables and cereals are also the main sources of spermidine. The main origin of dietary spermine is meat and fish, except in Sweden, where it is vegetables and cereals.

At present there are no official recommendations for the daily intake of polyamines, but some suggestions have been made. According to proposed an intake around of 540 μmol/day, taking into account the guidelines of a healthy diet that promotes a high consumption of fruits, vegetables and cereals. In Asian countries, Polyamines daily per capta and amount per 1000 kcal of three polyamines. Which is predicted to be 106 μmol/day, 91 μmol/day, 38 μmol/day, respectively. Putrescine, spermidine, spermine. (35,36)

Regulations Policy

The toxicity of PA's is a very important parameter, however, determination of the exact toxicity difficult task, due to effect does not depend only on PA's presence alone, but also influenced different compounds. In addition, toxic effect of PA's on organism is also dependent by individual to individual. It state's that PA's toxicity will depend on two factors (quantitative and qualitative) and related to the consumer (individual susceptibility). As there is no legal limit stated. In fact, concentration level of histamine is listed by law in some type of food product (fish food). According to the data from food intoxication a legal upper limit of 100 mg histamine·kg $^{-1}$ food and 2 mg·L^{-1} of ethanol has been suggested. The upper limit accepted histamine levels in fish were also established in other countries. For example, the European Union has established regulations according to which histamine level should be below 100 mg·kg^{-1} in raw fish, and below 200 mg·kg-1 in salted fish. In USA, the Food and Drug Administration,

established a maximum limit of 5 mg histamine/ 100 g product at the port and 10 mg histamine/ 100g product in pickled fish for species susceptible to form histamine. A recommended upper limit 26 of 100 to 200 mg kg−1 for histamine in meat products has been proposed by the Netherlands. There are no established standards for cadaverine, putrescine or other BA's, only some recommendation are given, for instance, the recommended maximum level of tyramine has been in the range of 100-800 $mg \cdot kg^{-1}$ of food. Value of 30 mg·kg-1 for β-phenylethylamine has been reported as toxic dose in food. (36,37,38,39,40)

Physiological and Toxicological Aspects of Polyamines

Many PA's are important for normal function of biological systems. for instance, in eukaryote cells they're important precursors for a spread of precursors, some play an important role as neurotransmitters. Putrescene and spermidine are involved in critical biological functions. However, in larger concentrations, usually from an accumulation during a food source, these compounds are often toxic. Biogenic amines could also be a constituent of the many foods and consumption are often problematic for human health. High PA's concentrations can cause flushes, headache, nausea, palpitations of the guts and changes in vital sign and lots of other physiological problems. Important PA's found in food include histamine, tyramine, putrescene, cadaverine and phenylethylamine. Polyamines, like putrescine, cadaverine, agmatine, spermine, and spermidine, are naturally present in food and are involved in growth and cell proliferation. The toxicological effects of the many of those compounds has been reported for several reasons including the effect they will affect human health. Many of those amines within the presence of nitrites are often potential carcinogens when converted to nitrosamines, but nitrosamines formed from the polyamines only become a health risk. (40,41,42,43,44)

Potential Effects of Polyamines

Aging

As the age increases the cellular level of spermine and spermidine and therefore the enzymatic activity decrease. Improvement of the diet with polyamines during this stage can reduce the danger pathologies and promote long-life. study in aging mice, a diet with high levels of spermine and spermidine (374 and 1,540 nmol/g, respectively) increased the concentrations of those compounds within the blood and reduced levels

of pro-inflammatory markers, age-associated DNA methylation, renal glomerular atrophy and mortality. it's also been observed that spermidine increases autophagy, which involves the removal of damaged proteins and organelles from cells, thus inhibiting the aging process. during a follow-up study of a cohort of 829 participants during 20 years, spermidine showed the strongest inverse relation with mortality among 146 nutrients investigated. This effect was dose-dependent, and therefore the authors explain that spermidine effectively induced autophagy and may reduce the acetylation of histones, which are critical processes for cell homeostasis in aging. during this sense, a diet rich in spermidine, mainly from foods of vegetable origin (green pepper, wheat grain, mushrooms, etc.), was related to a decrease within the risk of all-cause mortality within the general community. (44,45,46,47,48)

Cardiovascular disease

The antioxidant and anti-inflammatory effects to polyamines can play a vital role within the prevention of chronic inflammatory pathologies, like cardiovascular diseases. a better intake of spermidine has been correlated with a lower incidence of cardiovascular diseases and a decrease in vital sign and coronary failure. it's likely that the anti-inflammatory role of polyamines within the prevention and treatment of disorder is analogous thereto of polyunsaturated fatty acids (PUFA 3-n) and statins. In animal studies, of aging mice, spermidine has seen that decreased age-induced arterial stiffness and oxidative damage of endothelial cells. additionally, 6-week supplementation of spermine and spermidine in mice reversed age-associated changes in myocardial morphology (myocardial fibrosis) and inhibited cellular apoptosis of the guts. (49,50,51)

Diabetics

Glycation has a crucial role within the development of diabetes complications. thanks to their chemical structure, polyamines could function as antiglycan agents, delaying the buildup of advanced glycation end-products (AGEs). This effect would flow from to the interaction between the free amino groups of polyamines and therefore the highly reactive carbonyl compounds. In vitro studies have demonstrated that the millimolar concentrations of spermine present within the nucleus can protect DNA and histones from glycation. On the opposite hand, some authors have observed a better PAO activity in children with DM type 1, which could induce an increased production of free radicals and subsequent oxidative damage. Therefore, more studies are needed to clarify

the role of polyamines in diabetes and establish recommended levels of polyamine intake for the diabetic population. Elevated levels of polyamines in cancer patients are related to tumor growth. A deregulation in polyamines biosynthesis, mainly thanks to a rise within the activity of the ODC enzyme, leads high intracellular polyamine content in cancer cells. Therefore, controlling polyamine synthesis might be useful in antineoplastic therapy. (52,53)

Cancer

An increase of the acetylated metabolites of polyamines has been observed in urine or blood in patients suffering cancer disease. The increase of acetylated polyamines in urine could also be explained by a rise of cellular polyamines, a rise of the SSAT activity, a serious excretion of acetylated metabolites from cells or by a decrease of their oxidative degradation by PAO enzyme, although the molecular mechanisms aren't well-elucidated. The event of more sensitive metabolomic techniques within the last decade has allowed detailed polyamine metabolic profiles to be related to certain sorts of cancer. In fact, increased levels of acetylated polyamines in urine or blood, particularly, N1N12-diacetylspermine, N1N8-acetylspermidine, N1-acetylspermine, and N8-acetylspermidine are found in patients with ovarian, prostate, colorectal, pancreatic, breast and lung cancers. Among them, N1N12-diacetylspermine has been extensively described because the best urinary biomarker for several sorts of cancer and to watch tumor's progression. (55,56,57)

Polyamines and Health

Polyamines are important for cell maturation and proliferation, DNA stabilization, RNA transcription, protein synthesis, immune response modulation, apoptosis, ion channel regulation, notably potassium channel blockage, and antioxidant activity. Polyamine’s antioxidant activity is primarily influenced by membrane lipids and nucleic acids. Because of its increased amount of positive charges, spermine is the polyamine with the strongest antioxidant effects. Metal chelation, which limits the creation of hydroperoxides and delays the generation of secondary oxidation products, is the major mechanism of polyamine antioxidant action. Polyamines have also been suggested as a way to remove free radicals, particularly in lipophilic media. (58,59,60)

Analytical method (Example milk sample)

High performance liquid chromatography.

Polyamines standard

(putrescine, spermidine, spermine)

Internal standard

(1,7-diaminoheptane), perchloric acid 70%, benzoyl chloride 99%, and acetonitrile 99% analytical grade.

Extraction of polyamines

add 3ml of 0.6 perchloric acid to 5ml of sample, and spiked with 2µM internal standard (1,7-diaminoheptane).

This mixture was kept for at 4°C to separate the protein phase from acidic extract phase including polyamines.

Polyamine derivatization

the acidic extract of polyamines with small amount of 2N NaOH to keep the pH above 13.

Procedure

This following mixture was used for derivatization process by adding 10 µl of benzoyl chloride. Further the solution was concentrated by evaporation with nitrogen gas. The residue benzoylated polyamine was dissolved in 1ml of 38% acetonitrile in water.

Following solution was filtered using syringe filter. Finally injected into the High Performance Liquid Chromatography equipped with Nova-Pak C18 column (15×3.9 mm) and Waters UV detector 996.The entire run was under isocratic elution with flow rate of 1ml/min.

Results

benzoylated polyamines were detected by UV absorption at 198nm.

Polyamine observed based on retention time of polyamines standards.

What are the existing reported techniques for polyamines?

Methods used for polyamine detection:

A) High performance reverse phase liquid chromatography (HPLC)-fluorescence detector.

B) HPLC-fluorescence detector;

C) HPLC with cation exchange resin;

D) Micellar electrokinetic capillary chromatography;

E) Atmospheric pressure chemical ionization-mass spectrometry (MS);

F) Ion-pair reverse phase HPLC-fluorescence detector;

G) HPLC-UV detector

Fluorescence detection

When Wine sample were analyzed which gave results as there was no difference in wine produced by selected microorganism and those which were present on the skin of grapes. This experiment was conducted by using pre-column OPA derivatization and HPLC and the separation was carried out using C18 column at 23 C. Potassium dihydrogen phosphate and disodium hydrogen phosphate adjusted at a pH of 7.2. The sample elution was performed in stages, with a flow rate of 1 mL min1, and a solvent gradient elution programmed was carried out.

An ionic liquid based ultrasonic-assisted liquid–liquid micro extraction (ILDLLME) method for determination of BA's in beer samples which was developed with the combination of HPLC-FL. DMQC-Osu was used for derivatization of BA's. By the help of ionic liquid helps us in reducing toxicity and provides us better results and obtain single-step analyzable extract.

A HPLC method with pre-column derivatization by OPA and NAC mixture were used for determining 24 amino acids and PA's in grape juice, wine, honey and physalis fruit. OPA and NAC are used to provide a stable derivative. Lower limit of quantitation (LLOQ) was found as 100 g/L for tyrosine, phenylalanine, putrescine and cadaverine.

The ultra-high pressure liquid chromatography (UHPLC) method coupled with an on-line OPA post column derivatization were described to determine 12 BA's and polyamines in different food matrixes (wine, fish, cheese) in a single chromatographic run for 7-min elution programmed. C18 column was used at 42 C while the post-column reaction equipment was kept at room temperature. Fluorimetric detection was carried out at 340 nm for excitation and 445 nm for emission. Detection limits were found lower than 0.2 mg L1 and a determination limit falling below 0.3 mg L1 for all amines. (69)

Mass spectrophotometric detection

HPLC atmospheric pressure chemical ionization MS (HPLC– APCI–MS) method was developed for the determination of 8 BA's in donkey milk samples. Donkey milk was treated to get rid of proteins, pre-column dansylation of the amines were administered.

The derivatization was administered within the dark for an hour at 50 C. HPLC was utilized in Reverse Phase mode. Separation was performed with a flow of 1 mL min-1 and putrescine, spermine and spermidine were eluted in but 20 min. (La Torre, Saitta, Giorgia Potortì, Di Bella, & Dugo, 2010).

Ultrahigh-performance hydrophilic interaction chromatography (UHPLC-HILIC) including orbitrap MS for detection were developed to determinate eight BA compounds from canned and frozen tuna. Tuna samples were extracted with matrix solid-phase dispersion using CN-silica sorbent and eluted with a mix of aqueous ammonium format buffer and acetonitrile. Instrumental conditions include an injection volume of 5.0 lL, a column temperature of 30 degrees, and a flow of 0.75 mL min1. Mobile phase was ammonium format buffer and acetonitrile (Self, Wu, & Marks, 2011). (70)

Why should there is a need to switch to biosensor

The analytical methods to determine polyamines in food are mainly based on the chromatographic separation coupled with distinct detection techniques due to their high resolution, sensitivity and versatility. Gas chromatography, thin-layer chromatography and high-performance liquid chromatography have been applied for the analysis of polyamines in food. The high or ultra-high-performance liquid chromatography with ion-exchange columns or reverse-phase columns to separate polyamines are the most frequently this the majorly used analytical techniques. There are few more techniques which are coupled with other chromatographic methods. Such as the ultra-violet, fluorescence and mass spectroscopy techniques. Polyamines have low absorption coefficients or quantum yields and require derivatization when the method involve UV or fluorescent detection. This includes extensive cost for processing the sample. For which there is search for lesser and cost effective method.

Electrochemical sensors or biosensors is an analytical procedure which is less expensive, less time-consuming, and analytically simpler, especially for routine screenings. This method is used for primary screening technique. Electrochemical biosensors usually consist on immobilized enzymes, which catalyze the oxidative deamination of polyamines present in foods, and a working electrode that detects the production or the consumption of the redox species produced by the enzymatic activity.

Different electrochemical sensors developed for the rapid determination of polyamines in food showed low detection limits and good selectivity toward these compound.

Basic concept of a PA's biosensor

Biosensors are an easy, robust and economical analytical devices supported the principle of the interaction of biological elements to a particular analyte (substance to be detected within the sample) and therefore the generation of physicochemical changes (such as transfer of electrons or heat, change in pH and mass, absorption or release of particular ions or gases). Basically, biosensors contain bio recognition layers (formed by antibodies, nucleic acids or enzymes), physical transducers and processors. Biological components recognize specific target analyte and therefore the transduced signal becomes amplified, processed and converted into a digital format for the observation. The transducer, being a crucial element within the biosensor device, transforms the resulting biochemical signal into a quantifiable signal. The signal produced is proportional to the concentration of the target analyte present in its proximity. Amongst the presently available biosensor systems for PA analysis, electrochemical biosensors hold leading positions for high performance, simple design, rapid screening methods, low cost, low detection limits and therefore the possibility of miniaturization. especially, enzymatic aerometric biosensors have attracted enormous attention in electroanalysis during the last decade. These devices unite the enzyme selectivity for the identification of a selected target analyte with the direct transduction of the speed of the biocatalytic reaction into a current signal, enabling rapid, sensitive and accurate detection. Thus, biosensors have gained huge attention within the electroanalysis of PA's during recent years. Biosensors have now proceeded to field testing from laboratory levels and lots of are commercialized. the present review provides comprehensive data about PA's biosensors.

Mechanism involved in enzymatic detection of PA's

The enzymatic PA's biosensors based on the incorporation of an enzyme in close proximity with the biosening electrode. The enzyme either inhibit an electroactive reactant or produces electroactive species during catalysis and the analyte can be measured directly by the generation or depletion of reactants. Amine oxidases (AOx) are a class of enzymes that play the chief role in quantifying PA's. Therefore,

understanding different enzyme immobilization technique and selecting the specific one option among them is of prime importance. (61)

Methods of enzyme immobilization

IF we require to produce a viable enzymatic biosensor then enzyme must compactly have attached with a working electrode without denaturing. This important step of enzyme immobilisation decides the level of sensitivity, stability and performance of the biosensor. Various physical and chemical interactions have been described for immobilization of enzymes on different supports. (62,63)

Physical adsorption

The simple method of immobilisation in which enzymes are physically adsorbed onto an inert support by mild forces of attraction, primarily Van der Waals forces, ionic and hydrophobic interactions, salt linkages, and hydrogen bonding, resulting in the enzyme's stabilisation with no problems. It allows reversible enzyme immobilisation by placing enzymes on a transducer's particular membrane surface. Physicochemical characteristics such as pH, temperature, and polarity play a role in their interaction. As a result, when the enzymatic characteristics have degraded, contact can be reversed by manipulating the solvent conditions.

Pros: simple, cost-effective, and manageable. Easily binding and good enzymatic characteristics.

Cons: Desorption due to weak contacts, slower response time, and poor operational stability are all disadvantages. (64,65)

Covalent bonding

The commonly used technique for immobilization. It provides irreversible immobilization where a covalent bond is created between the enzyme functional group and chemical groups of the transducer establishing a stable covalent linkage between them. Covalent association is implicit by two ways- either by activation of the support by providing some reactive functional groups or by producing an activated group as a result of polymer modification. This method provides a wide range of options to select a carrier material due to its diverse functional groups. Some parameters like low ionic strength, shallow temperature and a physiological pH is needed for covalent bonding.

Thermal stability and half-life of enzymes can be enhanced by linking other supports (like chitosan, silica, etc.) with covalent coupling.

Pros- provide stability, quick response, no enzyme leakage, reduced diffusion barriers various supports are available with different functional groups.

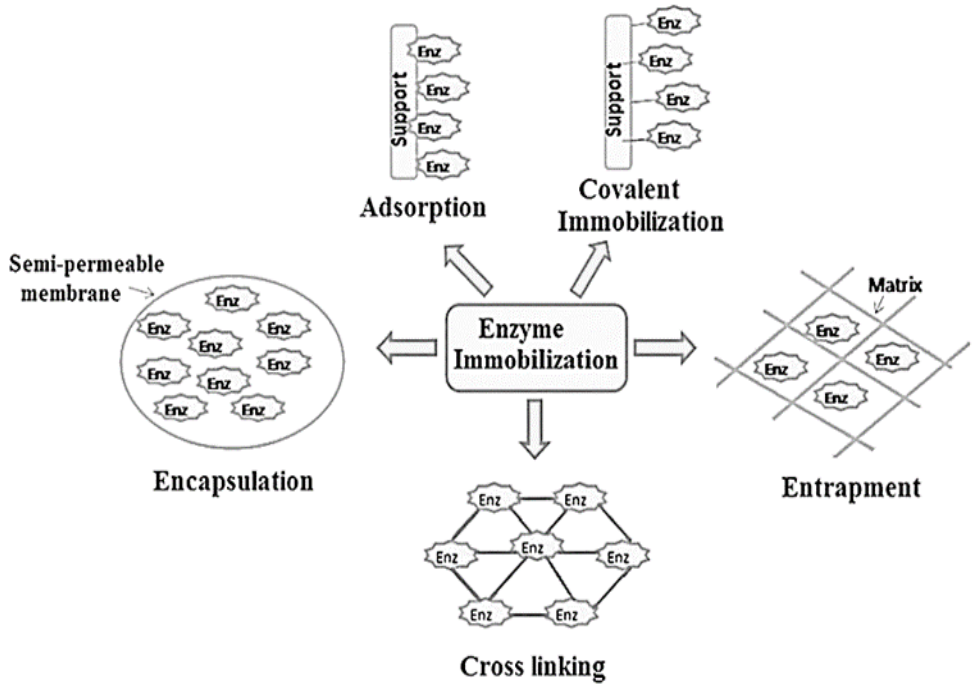
Cons- chemical modifications may result in enzyme denaturation. (66,67)

Cross linking (Co-Polymerization)

The method of enzyme immobilization does not require support or matrix and irreversible. Different methods of enzyme immobilization. 7 immobilizations are acquired by direct cross linking between enzyme and the transducer with the help of polyfunctional reagents. Glutaraldehyde with BSA is that the most widely used linkage among various cross linking agents because it is economically favorable and simply accessible. The absence of support provides preventions of all the hindrances related to it making it a serious characteristic of this method.

Pros- simple and price effective, very less desorption.

Cons- agents used may denature the enzyme, requires a pure sort of the enzyme with high activity. (68,69)

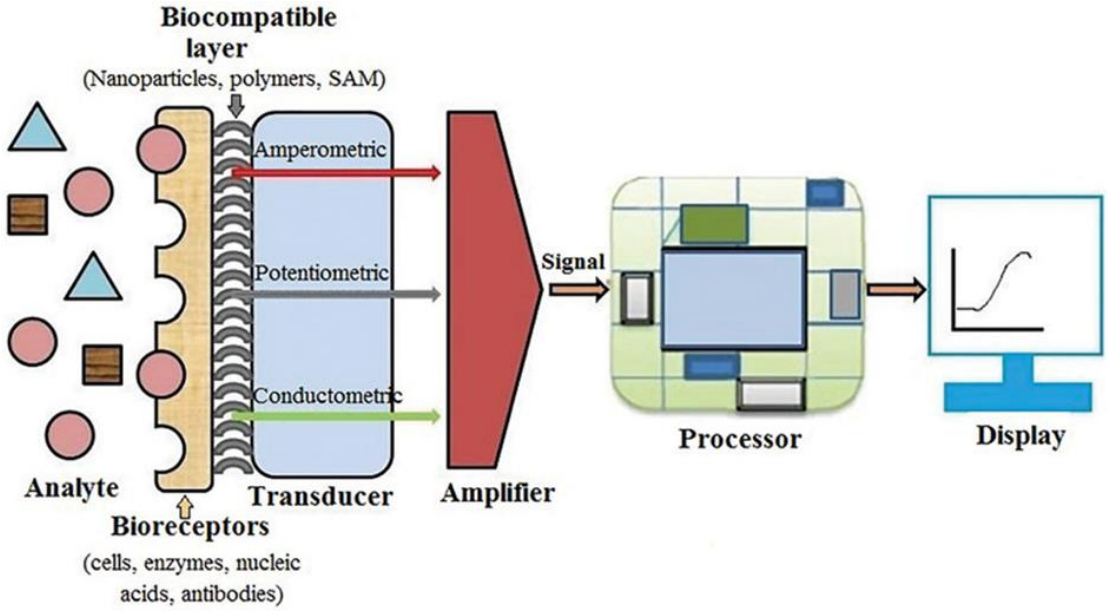


Feature no. C

(https://doi.org/10.1080/07388551.2019.1680600)

Methods for the determination of polyamines by biosensor

Analytical methods for the determination of PA's are mainly supported chromatographic separations using high-performance liquid chromatography (HPLC) or gas chromatography (GC) followed by the detection by mass spectrometry. Alternatively, upon pre- or post-column derivatization, UV and fluorescence spectroscopy detectors are commonly integrated with chromatographic methods. Actually, a chemical derivatization is mandatory to permit the sensitive determination of PA's by spectroscopic techniques. Thin layer chromatography, NMR and colorimetric methods, were also proposed. Sensor and biosensors represent competitive options to those conventional analytical methods. A sensor is consisting of three main elements: a receptor, a transducer and an electronic component.



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(https://doi.org/10.1080/07388551.2019.1680600)

The receptor recognizes the analyte during a complex matrix resulting in a chemical recognition event. The transducer converts the chemical recognition event into a detectable electric signal for the subsequent instrumental Endogenous and food-derived polyamines: determination by electrochemical sensing processes. The electronic component filtrates, amplifies and operates the electrical signal to optimize the ultimate readout. The sensor transduction systems can operate consistent with different physical principles. Sensing devices supported calorimetric, gravimetric, optical, and

electrochemical transducers are reported. An electrochemical sensor, is predicated on the presence of an electrochemical transducer, transforming the analyte-sensor interaction in an electrochemical signal. Three different electrochemical transducers are often found in literature: aerometric, potentiometric, and impedimetric systems. Aerometric transducers are ready to transform the concentration of the substance under investigation (analyte) in an electrical current. Alternatively, potentiometric transducers are dedicated to the determination of a measurable potential or charge accumulation. The transducers determine the conductive properties of a medium as a function of analyte concentration. When the electrochemical sensing platform is provided with a minimum of one biological component, then it's defined an electrochemical biosensor. The biological component, defined bio element, results in a biological event or reaction cascade within the presence of the analyte, which may be followed by the sensor transducer. This element produced a final signal proportional to the concentration of the analyte. The biological component is often represented by an enzyme, an antibody, a DNA sequence, an aptamer, a recognition protein, a membrane receptor or maybe intact Cells. Many electrochemical biosensors of PA's are simply supported immobilized amino oxidases (AOs), which give the biocatalytic oxidation of PA's. The enzymatic reaction generates peroxide, which is detected at a working electrode. Some AOs allow the event of versatile electrochemical biosensors and located applications during a sort of food samples. Bioenzyme biosensors also are very fashionable and involve the biocatalysis of the enzymatic products of AOs by a second enzyme. The working electrode will finally detect the assembly or the consumption of the redox species produced by the enzymatic activity of the second enzyme. (68)

Biosensors for the determination of polyamines in food

The knowledge of PA content in food would be important to modulate the diet for a specific human health condition. However, to avoid the risk, the current tendency of the agro-food industry is to launch products with low PA's levels on the market. However, no upper limit of PA levels in food is in place, the amount of PA's in food is highly uncertain, ranging from Nano moles to micro-moles per gram, depending on the quality of the food, on environmental conditions and on the possible existence of microorganisms involved in food decay. In fact, PA's can be originated from the activity of food-borne bacterial enzymes catalyzing the decarboxylation of amino acids. Diamine oxidase (DAO) has been used for detection of PUT and CAD in vegetable,

fish and meat samples. Putrescine oxidase (PUO) for PUT in fish, meat, alcoholic beverages, animal plasma, and human blood. Polyamine oxidase (PAO) for SPD and SPM in fish, vegetables, human urine and blood. Spermine oxidase (SMO) for SPM and SPD in human blood and agmatinase (AUH) for AGM in mollusc samples. Other electrochemical sensing applications were based on Bioenzyme biosensors. These biosensors put together mainly with horseradish peroxidase in combines with an amine oxidase. As an example, these two enzymes were co-immobilized on the surface of a graphite electrode with an osmium-redox polymer as electrochemical mediator. Mono-enzyme and Bioenzyme put together for both attractive options and helps us providing comparison study, which includes two alternative designs keep constant DAO as bio-recognition element. In the first case, DAO was directly immobilized onto a platinum working electrode (poised at + 700 mV). further, the enzyme combined with horseradish peroxidase into glass beads into a FIA reactor for using a glassy carbon working electrode. Interestingly, both biosensors displayed low detection limits, similar selectivity toward PUT, CAD and SPM and were successfully applied for the detection of PA's in gilthead bream sample. (70)

Biosensors for the determination of polyamines in fish

Environmental conditions and duration of the storage period before processing affect fish freshness, and therefore the rate of staple spoilage depends on the fish species and on the degree of microbiological contamination. Fish spoilage is amid protein hydroxylation into peptides and free amino acids, which are further degraded to thermally stable biogenic amines and polyamines. Thus, their levels in fish are potential indicators of food spoilage. Among the variability of PA's in fish, only CAD and PUT are identified as reliable markers of fish safety and quality and were proposed as indicators of fish freshness using different indexes or computational methods. It worth mentioning that CAD and PUT were suggested to potentiate the toxic activity of histamine by inhibiting the intestinal histamine-metabolizing enzymes, like diamine oxidase and histamine N-methyltransferase. Early samples of biosensors for fish quality assessment were proposed within the 1980s, but significant developments were reported only within the 1990s. A biosensor supported diamine oxidase (DAO) purified [2]from porcine kidney and immobilized on a nylon membrane by glutaraldehyde for measuring CAD and PUT in fish during storage. The enzyme membrane was fixed onto a Pt working electrode and therefore the H_2O_2 produced by the enzyme catalysis was

measured at +0.4 V with reference to an Ag/AgCl reference electrode. Several publications supported putrescine oxidase (PUO) as convenient bio-recognition system. The analytical specificity was studied toward PUT, CAD, SPD and AGM, and both biosensors were successfully applied to gauge fish freshness alongside storage. These electrode configurations were applied for the detection of PA's in mackerel and codfish. A Bioenzyme biosensor working within the absence of an electrochemical mediator was proposed for AGM determination as indicator of squid freshness. during this case PUO was utilized in combination with agmatinase (AUH). AGM was converted to AUH and therefore the as produced PUT was further oxidized by PUO. the quantity of AGM decided by the quantity of oxygen consumed by an oxygen sensor. (71)

Biosensors for the determination of polyamines in plant samples

PUT, SPD, and SPM are the most frequent PA's in plants, and they're all involved in a variety of biological activities like cell division, cell elongation, embryogenesis, root formation, floral and fruit development and ripening, pollen tube growth and senescence, and stress response. PUT, CAD, SPM, and SPD are almost common in all vegetables and fruits, with concentrations around a few mg/100 g fresh weight, according to various research. A unique biosensor configuration was created to track changes in amine concentration throughout the ripening of sweet cherry and apricot fruit in a changed atmosphere. The biosensor system included an AO mounted onto a polymeric membrane as a bio recognition element, as well as a Pt electrode for detecting the enzymatically generated compounds. (72)

Biosensors for the determination of polyamines in meat

The concentration of PA's varies depending on the type of meat, and recently evaluated common levels by kale (2014). PUT and other amines have been linked to microbial development and meat rotting in general. Even if production processes, packaging techniques, additives, and storage circumstances may vary the PA content, inner organs and metabolically active tissues (liver, kidney, spleen, etc.) have high SPM levels. By using glutaraldehyde chemistry to encapsulate PUO on a platinum electrode, a biosensor for detecting meat freshness and quality via potential-step chronoamperometry was created. To prevent electrode fouling, a nafon membrane was attached to the sensing surface. Furthermore, PUO was connected to chitosan beads by glutaraldehyde and used to detect PUT, CAD, and SPD simultaneously. (73)

Biosensors for the determination of polyamines in dairy products

PA concentration in milk is lower than in cheese, this is stated to take advantage of yield, lactation period, and cow type. Various factors affect the PA content of cheeses (milk protein content, bacteria occurrence, thermal treatment, and storage conditions), and the overall PA level is higher in aged cheeses than in fresh cheeses. Biosensors for PA detection in dairy products were only mentioned in a few articles. The total biogenic amines in different cheeses were determined using DAO from lentils immobilized on a polymeric membrane and paired to a platinum working electrode. Another example was an FIA aerometric arrangement made up of a Pt (or Au) working electrode and commercial DAO entrapped onto an electro synthetized film through glutaraldehyde. The system exhibited exceptional stability, sensitivity to PUT and CAD, and the capacity to inhibit a specific electroactive interference. The anti-fouling and anti-interference activity of the electro synthetized film was investigated as a function of the number of layers that make up the film. There was also a proposal for an enzyme-free electrochemical sensor by Sun et al. (2003) used a combination of capillary electrophoresis and pulsed aerometric detection (PAD). PA's in milk can be detected using this approach. PUT, CAD, SPD, and SPM were well resolved under optimal conditions. (74,75)

Biosensors for the determination of polyamines in alcoholic beverages

Alcoholic beverages, such as wine and beer, are examples of fermented goods that may contain substantial levels of PA's. PUT and AGM are the most common PA's in wine, and they're formed by decarboxylation of free amino acids during spontaneous malolactic fermentation. Many factors, including grape varietal, amino acid concentration, ageing, and wine making processes, influence their ultimate content in wine. PA's were also discovered in beer, though in lower proportions than in wine. The barley variety used in the brewing process, malting technology, wort processing, and hence the fermentation conditions have an impact on the PA level of beer. (76)

Biosensors for the determination polyamines in cancer

The increased PA content in neoplastic cells compared to normal cells has been observed in numerous publications and reviews. Furthermore, the addition of PA's to normal tissues has been shown to stimulate the growth of cancer cells. Polyamine signaling, engaged in cancer in multicellular systems. Several cancers have been linked

to PA's as biomarkers, including breast cancer, lung cancer, prostate cancer, and head and neck cancer. There are just a few articles in the literature that reported on the electrochemical determination of PA's in human tumors tissues. For the detection of PA's in crude human liver extracts, bovine serum amine oxidase (BSAO) was immobilized on iron oxide nanoparticles that had previously been coated with a chromate ion shell and then integrated into a carbon paste electrode. The biosensor was able to distinguish between tumorous and healthy tissues. On animal tumors tissues, some electrochemical sensors for the detection of PA's were tried. Chromatography was used to separate PA's from rat brain homogenates. PAO as a bio element was identified in a post-column electrochemical reactor. Alternatively, HPLC was used to isolate SPM, SPD, PUT, and CAD from rat brain homogenates, and an enzyme less electrochemical sensor was used to quantify them. (77,78)

Conclusion

My work comprises of review of polyamines and its following related development. This work includes the relationship between the PA's and its development between plants and animals. Now a-days its increasing popularity toward endogenous PA production via genetic manipulation to regulate plant and animal development. This work shares us information that how polyamines are important in physiological functioning and human health. As mention above several study's shares information about polyamines being important in different stages of life. Such as the aging when requirements are high. The antioxidant and anti-inflammatory effects described that polyamines play important role in preventing chronic diseases foe example cardiovascular and diabetics. This also in case of cancer.

The polyamines contents in food, variable in different type of food items. As mention above breast milk provides the first dietary exposure to these compounds. Further plant derived food items have the highest content of spermine and spermidine polyamines. Whereas putrescine levels are found high in vegetables and fruits. In animal derived food items the polyamines content is also high. The wide range of putrescine contents can be explained by decarboxylation activity of spoilage or fermentative bacteria. A few study's estimating polyamines intake are possibly highly variable. As further in the going through the article we discus about the identification of polyamines through various methods using different analytical methods.in the end the article discus about the new biosensor development towards the identification of polyamines for Following

sample such fish, meat, dairy products, alcohol sample. Biosensor were also derived for determining cancer as the level of polyamines varies between a healthy individual and normal individual. This work has done extensive research on polyamines produced by various plants and animals. Also provides information about the detection of polyamines through various analytical tools. Finally provides information about the biosensor developed for detection of polyamines in various entities.

List of abbreviations used

1) PA's = polyamines
2) Spm = spermine
3) Spd =spermidine
4) Put = putrescine
5) (Tspm) = thermospermine
6) (cad) = cadaverine
7) (PSCC-PA's) = perchloric acid-soluble covalently conjugated polyamines
8) (PISCC-PA's) = perchloric acid-insoluble covalently conjugated polyamine
9) (PISCC-PA's) = prichloric acid-insoluble covalently conjugated polyamine
10) (PUFA) = polyunsaturated fatty acids
11) (F-PA's) = free polyamines
12) (AGEs) = advanced glycation end-products
13) (HPLC) = High performance reverse phase liquid chromatography
14) UV = ultra violet radiation
15) (AOx) = Amine oxidases
16) (GC) = gas chromatography
17) (FIA) = flow injection analysis systems
18) (ILDLLME) = An ionic liquid based ultrasonic-assisted liquid–liquid micro extraction
19) (LLOQ) = Lower limit of quantitation
20) (UHPLC) = ultra-high pressure liquid chromatography
21) (HPLC– APCI–MS) = HPLC atmospheric pressure chemical ionization MS
22) (UHPLC-HILIC) = Ultrahigh-performance hydrophilic interaction chromatography
23) (DAO) = Diamine oxidase
24) (PUO) = putrescine oxidase
25) (PAO) = polyamine oxidase
26) (SMO) = spermine oxidase

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