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Nitrate in vegetables and fruits consumed in Sicily (Italy) by potentiometry and health risk assessment

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ABSTRACT

In this study, nitrate concentration of 81 fruit and vegetable samples consumed in several cities of Sicily region (Italy) were evaluated. After determining the concentration of nitrate, non-carcinogenic risk assessment in two consumers age groups (children and adults) were carried. The mean concentration of NO_3^- in products consumed in Italy was 613 mg Kg^{-1}. The concentration ranged from 23 to 4804 mg Kg^{-1}, the highest one was related to a rocket sample which, however, is lower than the legal limits. Although the matrix considered is much more complex than the aqueous ones, the results obtained by us show that the optimized method for the potentiometric determination of nitrates, using an ion-selective electrode, is accurate, precise and selective. All possible interfering substances, in particular chlorides, were eliminated by adding a silver sulphate solution to the suspension obtained from the sample treatment.

1. Introduction

It is well known that plant based food, especially fresh fruit and vegetables, in the diet ensure a part of the micro and macro nutrients necessary for the human body, resulting in positive effects on health, in fact, several World Organizations (UN, 2004) recommend a consumption of 400 g of these foods (excluding tubers) per day to prevent some chronic diseases (heart disease, cancer, diabetes, obesity, etc.). Compared to other foods, the consumption of vegetables, on the other hand, involves a greater intake of many hazardous organic compounds (pesticides, PAHs, etc.) (Amorello et al., 2022; Amorello et al., 2023) and inorganic pollutants (Barreca et al., 2023) including nitrates (Bahadoran et al., 2016).

The main source of nitrates for humans is the diet, mainly vegetables, because during growth, they need nitrogen for the synthesis of amino acids and proteins, but in the presence of a high availability of nitrogen compounds in the soil, they accumulate it as nitrate. For humans, specifically, they derive from vegetables (80 %), drinking water (15 %) and food products such as meat, cheese and cereals (5 %) ((EFSA). 2008). In these latter foods, nitrates and nitrites are added for their antimicrobial effect against *Clostridium botulinum* and the germination of spores and bacteria, furthermore, nitrate imparts the typical red colour to processed meats.

The nitrate content in vegetables varies based on different factors: use of nitrogen fertilizers, harvest periods, solar radiation, type of cultivation, temperature and soil characteristics. The ever-increasing demand of vegetables, both for food and industrial purposes, by world population, has led to an increased use of nitrogen fertilizers during the last fifty years from about 11 Tg N per year in 1961–108 Tg N per year in 2013 (Lu and Tian, 2017). This has led to an average increase in nitrate concentrations in agricultural products, in fact, concentrations from 1 to 10,000 mg Kg^{-1} have been quantified (Colla et al., 2018).

Nitrate ion is relatively harmless, but, in the organism, following the reduction catalysed by salivary enzymes and oral bacteria, reduced species such as nitrite ion, nitric oxide and N-nitroso compounds harmful to humans, are formed ((EFSA). 2008). The lethal dose by oral ingestion ranges from 67 to 833 mg Kg^{-1} of body weight (A. B. T. J. Boink, 1997), in particular, the lowest amounts are referred to children, elderly people and people with deficiency of cytochrome b5 reductase which converts methaemoglobin into haemoglobin. The acceptable daily intake (ADI), established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), is set at 3.7 mg Kg^{-1} of body weight

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(Organization., 2002).

Considering that fruit and vegetables are a significant part of the human diet, the risk of excessive nitrate intake on human health due to the accumulation of nitrate in this food cannot be underestimated. In order to establish any risks to consumer health related to nitrate intake through various sources, different analysis methods have been developed to quantify their concentrations. This need is important to monitor and protect the quality safety of food and water, in compliance with the limits established by the institutions. For this reason, it is necessary to monitor the quantity of nitrates present in foods, and consequently it is necessary to have reliable (accurate, precise, sensitive), simple, economical and fast analytical methods.

The choice of an analytical method to quantify nitrates must be made based on the type of matrix, their concentrations and the accuracy and precision required. Each method is subject to different interfering that can make it unsuitable for the chosen matrix or may require simple preparation of the sample or the use of reagents or solvents. Since nitrates are soluble in water, they cannot be quantified by gravimetric methods. The methods for quantifying nitrates are mainly divided into two categories: the first includes direct techniques that establish the concentration of nitrate, while the second includes indirect techniques in which nitrate is reduced to other species (nitrite, ammonium, nitrogen dioxide or nitric oxide). Most of the methods used are indirect methods, since nitrate is relatively inert unlike its reaction products. Considering the above, to quantify nitrates in complex matrices excluding methods that use expensive equipment, often not available in all laboratories, especially those intended for teaching, in this work we have optimized a potentiometric method using an ion-selective electrode. These sensors are extremely versatile and often used in the analysis of many food and environmental matrices, such as water intended for human consumption (Tang et al., 2012), river water (Goff et al., 2003), agricultural drainage (Goff et al., 2002), and foods such as meat (Choi and Fung, 1980), foods intended for children (Amorello et al., 2025; Pfeiffer and Smith, 1975) and vegetables. In this study, nitrate concentration of 81 fruit and vegetable samples consumed in several cities of Sicily region (Italy) were evaluated. After determining the concentration of nitrate, non-carcinogenic risk assessment in two consumers age groups (children and adults) were carried.

The Regulation (EC) No. 194/97 (Commissione Europea, 1997) is the first European Regulation on maximum nitrate concentrations in lettuce and spinach, while, the 2023 European Regulation 2023/915 (Europea, 2003), in addition to the previous vegetables, concerns rocket, cereal-based foods and those for infants and young children. The maximum concentrations (Table 1) differ not only for the type of product, but also for the harvest period, the type of cultivation and the post-harvest storage method.

Table 1
Maximum nitrate content for the commercialization of fresh vegetables.

Food	Harvest period/Crop type	mg Kg^{-1}
Fresh spinach		3500
Preserved, frozen or deep-frozen spinach		2000
Fresh lettuce excluding iceberg lettuce	**Harvest between 1**	5000
	October and 31 March:	4000
	Protected crops	4000
	Grown in open fields	3000
	Harvest between 1 April	
	and 30 September:	
	Protected crops	
	Grown in open fields	
Iceberg lettuce	**Protected crops**	2500
	Grown in open fields	2000
Rocket	**Protected crops**	7000
	Grown in open fields	6000
Cereal-based foods and other foods intended for infants and young children		200

2. Materials and methods

2.1. Instrumentation

The measuring apparatus consisted of a potentiometer (Model GLP 22) Crison Instruments (Italy). A double junction Ag/AgCl electrode, Crison 5044 was used as reference, while, a nitrate selective electrode (type 152223000 Mettler Toledo, Milano, Italy) was used as indicator electrode.

2.2. Chemicals

The standard nitrate solution (1000 ppm NO_3^-) was prepared by solubilizing 0.4073 g of KNO_3 (Sigma Aldrich, Milano, Italy) (previously dried in an oven at 105 °C for two hours and cooled in a desiccator) in 250 mL of deionized water. The solution obtained was stored in a polyethylene bottle. This solution was used to make additions to the samples. In detail, aliquots ranging from 0.1 to 2.5 mL, based on the initial estimate of the amount of nitrate or preliminary tests in the sample, were added to obtain a significant variation in terms of mV.

2.3. Quality assurance

The quantification of nitrate in the vegetable samples was preceded by the experimental determination of the RT/F ratio which appears in the electromotive force (e.m.f.) of Nernst Eq. (1), since during the potentiometric analysis, the temperature at which the measurements were carried out was almost constant but lower than 25°C, consequently, it was incorrect to use the value of the theoretical RT/F ratio (59.16).

$$E = E^* - \frac{2.303RT}{F} \bullet \log[NO_3^-] = E^* - 2.303b \bullet \log[NO_3^-] \quad b < 0 \tag{1}$$

In order to perform the method quality parameters, 10 successive additions of the standard nitrate solution (1000 mg L^{-1}) were made to 20.0 mL of blank, consisting of 20 mL of water, 0.1 g di Ag_2SO_4 and a.4 mL an $Al_2(SO_4)_3$ 0.9 M solution, measuring and plotting E Vs log [NO_3^-] (ppm) after each addition (Fig. 1).

The slope of the straight line obtained is equal to *b* which, in absolute value, represents the value of the Nernstian term in the experimental conditions. The high value of determination coefficient R^2 (0.9996) of the line confirms the good linearity of the method (Fig. 1). Fig. 1 shows the trend of the Gran function (G) (see Eq. (3)) as a function of the volume of nitrate solution added:

The nitrate concentration was given by:

$$[NO_3^-] = \frac{C_0V_0 + C_sV_s}{(V_0 + V_s)} \tag{2}$$

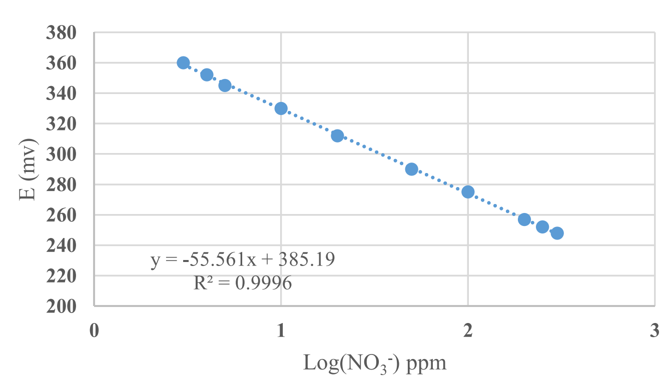

Fig. 1. Straight line for determining the RT/F ratio.

C_0 = initial nitrate concentration; V_0= initial volume; V_s and C_s are respectively the volume, and the concentration of the standard nitrate solution used.

$$G \quad = (V_0 + V_s) \bullet 10^{\frac{E}{b}} \quad = \quad (C_0 \quad V_0 \quad + C_s \, V_s \,) \tag{3}$$

where E is the e.m.f. measured (mV) after each addition. Gran linearization, or Gran's plot, is an interesting method to establish the analyte unknown concentration in potentiometric measurements (titrations) especially in the case of complex matrices. It is nothing more than the addition method applied to a nonlinear (logarithmic) relationship: Nernst equation. It requires little data to extrapolate the equivalence point. This method allows to use data from before (or after) the end point to localize the end point. In the case of standard additions, the plot of G is a function of V_s, by extrapolating the straight line on the negative x-axis, (G = 0) it is possible to calculate the nitrate concentration in the

cell (C_0) using the Eq. (4):

$$C_0 = \frac{C_s \, V^*}{V_0} \tag{4}$$

where V* represents the volume of standard nitrate solution required to have G = 0.

The linearity of the method was verified in the concentration range between 3 and 300 ppm of NO_3^- in the cell, starting from a solution constituted from distilled water (20 mL), 0.4 mL of a $Al_2(SO_4)_3$ 0.9 M and making additions of standards. Fig. 2 shows the variation of the potential difference with increasing concentration of nitrate.

The employed methods were texted for accuracy (recovery) by analysing several samples prepared by the authors: in the blender vessels, together with the water 50 mL, several volumes from 0.5 to 5 of a solution with known concentration (1000 ppm) of nitrate were added to known amount of samples (25 g), in which, according to preliminary texts, the nitrate concentrations were similar than the quantification limits, for example, a sample (25 g) of apples containing 23 mg kg^{-1} of nitrate was added with 0.5, 1 and 5 mL of standard (1000 ppm), obtaining concentrations of 42, 65 and 220 mg kg^{-1} of nitrate, respectively, instead of 43, 63 and 223 mg kg. Considering all the recovery tests carried out, on average it varies from 99 % to 103 %, which is certainly good enough for the purpose of this scope.

To establish the method precision, we repeated three times the analyses on different quantities of the same sample extending the test to seven different vegetables. In the Fig. 2 the values of the concentrations of the same samples are shown, reporting on the ordinate the value normalized respect to the mean value of the three measurements. The coefficient of variation (CV) of the method results lower than 5 %.

With regard to selectivity, a non-negligible interference is constituted by chlorides which are present in vegetable food in concentrations comparable and sometimes greater than those of nitrates (Pérez-Olmos et al., 1997). This interference was eliminated adding 0.1 g of Ag_2SO_4 to the suspension to precipitate the chlorides and eventually iodides, bromides, fluorides and thiocyanates.

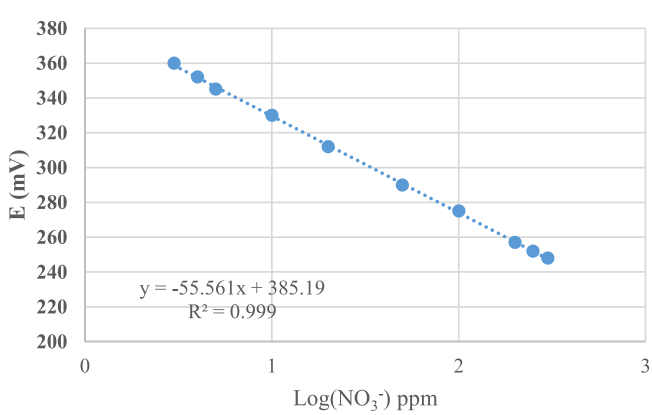

Fig. 2. Nitrate concentrations on different quantities of the same fruit and vegetable samples.

The detection limit (LOD) and the quantification limit (LOQ) were valued as reported in previous papers (Amorello et al., 2025; Di Gaudio et al., 2023; Orecchio et al., 2022).

LOD and LOQ were calculated as the mean value of seven blank signals (potential) plus the three- and ten-fold standard deviation of signals, respectively. Blanks were prepared in the same way as the vegetable samples. The initial weight of a sample, the final volume of the solution obtained and the slope of calibration curves were used to calculate LOD and LOQ values in mg Kg^{-1}

The LOD, referred to analysed suspension, is equal to 1.4 mg L^{-1} and the LOQ 2.3 mg L^{-1}. Considering the initial sample amounts and the dilutions, the LOD and LOQ values referred to the samples are 13 and 21 mg Kg^{-1}. All nitrate concentrations in the analysed samples were corrected for blank. For each sample, the analysis was repeated three times in order to define the confidence interval.

2.4. Samples

In this research, fresh and frozen fruit and vegetable samples were taken into consideration. After optimizing the analytical method, 85 vegetable samples (Table 2) originate in Sicily, from supermarkets, small traders or crops for personal consumption were analysed.

Some of the most commonly consumed foods, samples of the same vegetable were analysed from different distributors (shops, supermarkets, street vendors, etc.) to determine variability in their concentrations. Specifically, 10 tomato samples, 7 rocket salad samples, 7 lettuce samples, and 7 iceberg lettuce samples were analysed. The results show average values.

Simulating the real conditions of use, the fresh samples were analysed on the day of purchase or stored in the refrigerator (5°C) until the time of analysis (1–2 days) to limit changes in their composition. The frozen samples were stored in the freezer (-18°C) until the time of analysis.

2.5. Sample preparations

A weighed quantity of the edible sample (from 10 to 50 g) was

Table 2
Nitrate concentrations in the analysed fruit and vegetable samples.

Sample	mg Kg^{-1}	Sample	mg Kg^{-1}	Sample	mg Kg^{-1}
Apple	23	Courgette*	202	Peach	47
Apricot	57	Cucumber	69	Peas*	57
Asparagus	158	Eggplant	149	Potato	287
Avocado	98	Fava beans	95	Pumpkin	121
Banana	84	Fennel	202	Pumpkin flowers	323
Basil	1165	Figs	66	Pumpkin*	136
Beetroot	2814	Grapes	58	Radicchio	555
Belgian endive	332	Green beans	656	Red mulberries	123
Blueberries	93	Iceberg lettuce	1813	Rocket	4804
Blueberries*	164	Lemon	75	Spinach	2200
Cabbage	713	Lettuce	3323	Spinach*	1593
Carrot	507	Loquats	27	Strawberry	193
Carrot*	653	Mango	76	Sweet potato	265
Celery	2028	Mint	410	Swiss chard	2033
Cherries	76	Mushroom	85	Tomato	99
Chicory*	1139	Nectarine	45	Valerian	2164
Corn	87	Onion	65	White melon	48
Courgette	253	Orange	66	White mulberries	137

* frozen samples

homogenized in a blender, in some cases a known quantity of deionized water was added to obtain a sufficiently homogeneous suspension. According to the manufacturer's instructions for the nitrate ion selective electrode, interferences can be managed by adding silver sulphate, in our case to amounts between 5 and 20 g of the previously obtained suspension, 1 mL of the $Al_2(SO_4)_3$ solution as supporting electrolyte was added and brought to the volume of 50 mL.

2.6. Potentiometric analysis

To precipitate chlorides which if present in molar ratio (chlorides/nitrates) ≥ 1 would interfered (Toledo), 0.1 g of Ag_2SO_4 was added to the sample suspension. For each potentiometric determination, the temperature was measured, which however remained constant within ± 0.5°C from the beginning to the end of the measurements. Due to the complexity of the matrices, as in other papers (Amorello et al., 2025; Amorello et al., 2023; S. Orecchio & Amorello, 2019), the standard addition method was used for all vegetable samples: 4–6 additions from 0.5 to 5 mL of the standard nitrate solution (1000 mg L^{-1}) were made to a known volume of suspension. Plotting the Gran function, using the previously obtained value of the slope of the line (RT/F), against the volume of standard solution added a straight line is obtained whose intercept on the negative volume axis corresponds to V_s. From this data, the nitrate concentration in the suspension can be calculated. As examples, Fig. 3 shows the Gran plot for a lettuce sample (Fig. 4).

3. Results

A total of 81 samples of both fresh and frozen vegetable food were investigated for nitrate. The mean concentration of NO_3^- in products consumed in Sicily (Italy) was 613 mg Kg^{-1}. The concentration ranged from 23 to 4804 mg Kg^{-1}, the highest one was related to a rocket sample which, however, is lower than the legal limits, while in a sample of fresh spinach the limit (2000 mg Kg^{-1}) is exceeded (2200 mg Kg^{-1}). The results of quantifying the nitrate content in the same type of vegetable, sampled from different distributors (shops, supermarkets, street vendors, etc.) show that the standard deviations are between 19 % and 61 %, in particular: tomato (58 %), rocket (23 %), lettuce (61 %), and iceberg lettuce (19 %) (Figs. 9–12). The average values are shown in the results table.

The concentration of nitrates in the rocket sample is in good agreement with what has been reported by some authors (Hosseini et al., 2023). Considering all the investigated samples, a high standard deviation (160 %) has been found in the nitrate concentrations. The high

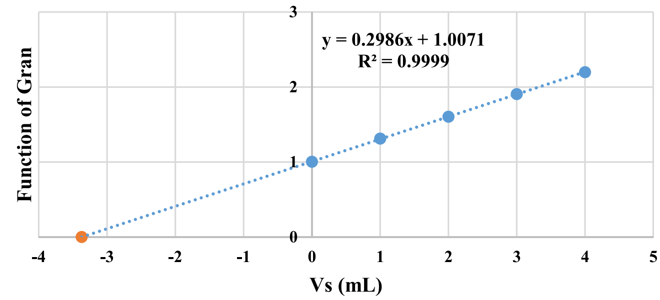

Fig. 4. Data relating an Iceberg lettuce sample.

variability of concentrations in the analysed fruit and vegetable samples can be attributed to their different characteristics, the type (Ebrahimi et al., 2020) of soil, the water used for irrigation and fertilization, in fact, the increase in population, the growing demand for fruits and vegetables, have caused an enlarged use of nitrogen fertilizers to intensify the agricultural yields, and this is one of the significant cause for the accumulation of nitrates in plant-based foods (Ebrahimi et al., 2020).

To allow a better visualization of the nitrate concentrations in the samples, the results were divided into three concentrations groups: < 100 mg Kg $^{-1}$ (Fig. 5); 100–1000 mg Kg $^{-1}$ (Fig. 6); > 1000 mg Kg^{-1} (Fig. 7). First group (Fig. 5) includes almost all the fruits, berries, cereals, legumes and mushrooms.

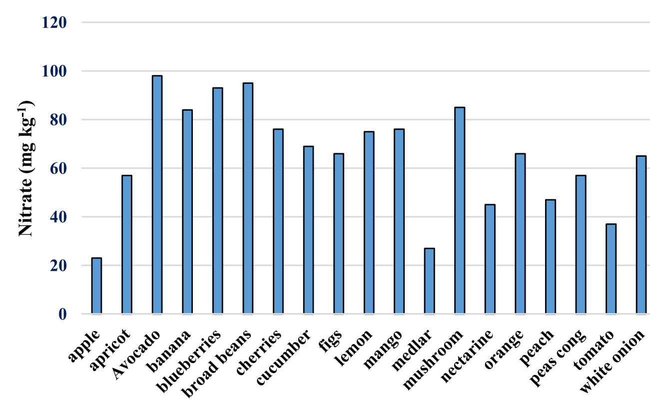

Fig. 5. Samples with nitrate concentrations less than 100 mg Kg^{-1} Second group (Fig. 6) includes most vegetables, tubers, roots and some fruits.

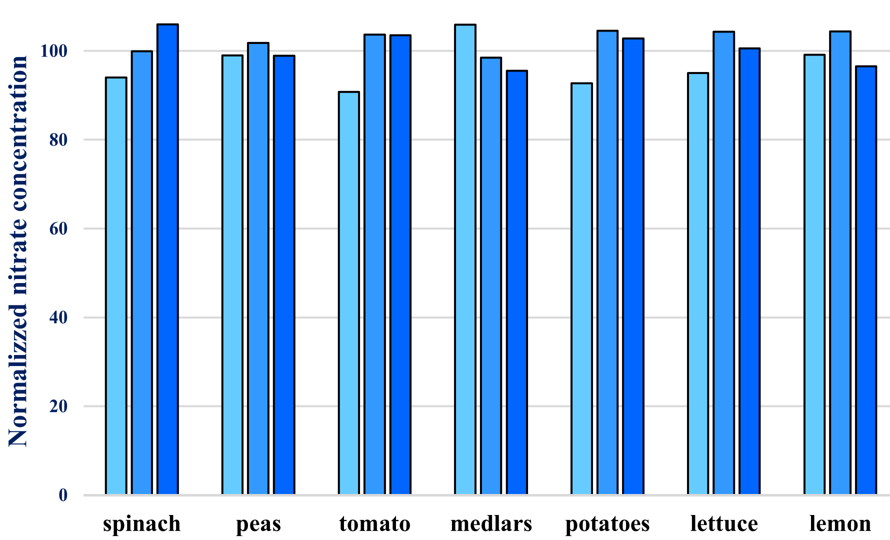

Fig. 3. Nitrate concentrations on different quantities of the same fruit and vegetable samples.

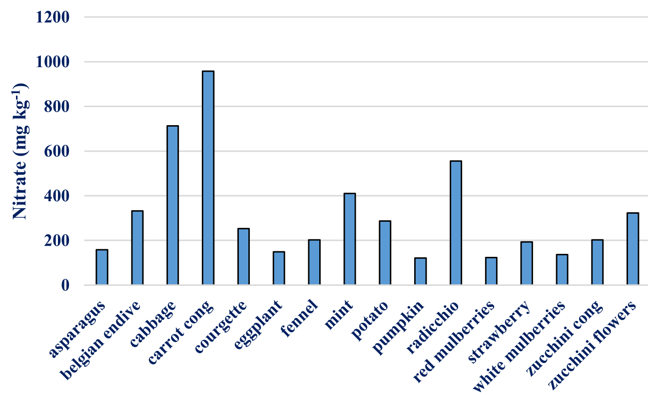

Fig. 6. Samples with nitrate concentrations 100–1000 mg Kg^{-1} The third group (Fig. 7) consists of green leafy vegetables and beets.

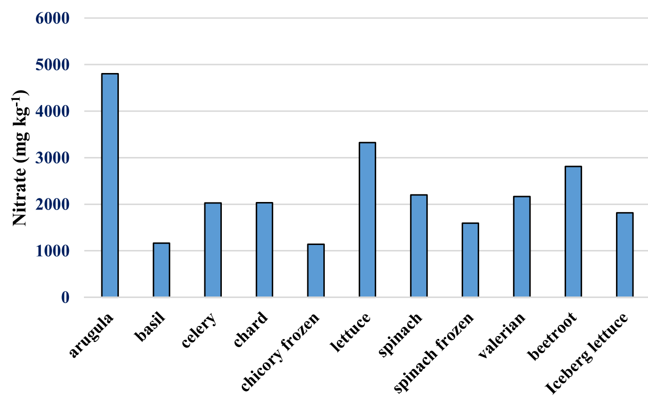

Fig. 7. Samples with nitrate concentrations > 1000 mg Kg^{-1}.

Further experiments were carried out by analysing fresh and frozen samples of the same vegetable: pumpkin, blueberries, courgette, spinach and carrots. The results (Fig. 8) show that for the samples of pumpkin, blueberries, courgettes, carrots there is no substantial difference between fresh and frozen product, while for spinach, the concentrations are quite different: 1593 mg kg^{-1} for the frozen ones and 2200 mg kg^{-1} in the fresh ones. In all cases, the above mentioned concentrations are lower than the legal limits for fresh spinach (3500 mg Kg^{-1}) and for frozen (2000 mg Kg^{-1}) (Europea, 2003).

Considering that several environmental and agronomic factors influence the concentration of nitrate in vegetables (Zhao et al., 2022), as already mentioned in paragraph 1.2.1., analysis were carried out on samples of the same food (tomatoes, lettuce, rocket and iceberg lettuce) but of different origin. The results of the measurements are reported in Fig. 9. Nitrate concentrations in tomato samples are in a wide range (37–206 mg Kg^{-1}). The mean value is 99 mg kg^{-1} and the standard deviation 55 mg Kg^{-1}, which corresponds to 67 %. This variation is in good agreement to literature for the same type of food, in particular,

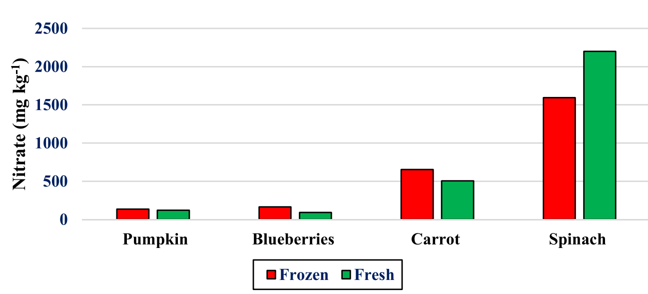

Fig. 8. Comparison between fresh and frozen samples.

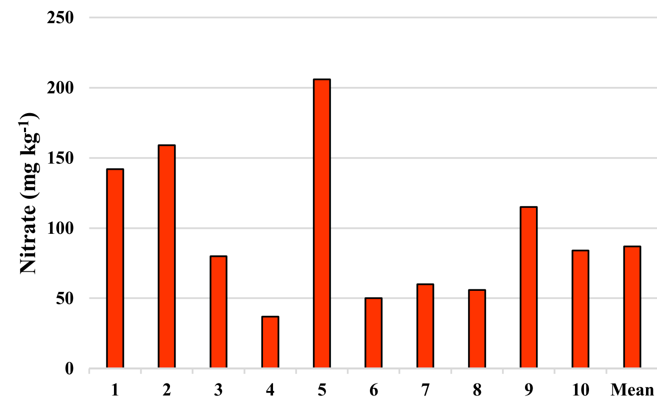

Fig. 9. Nitrate concentration in different tomato samples.

these show a range of concentrations from 5 to 392 mg Kg^{-1} (Abu-Dayeh, 2007; Colla et al., 2018) and from 0 to 58 (Figs. 10-13).

The nitrate concentrations of six lettuce samples range between 845 and 1371 mg Kg^{-1}, while one sample has a higher value (3323 mg Kg^{-1}), higher than the legal limit (3000 mg kg^{-1}). The average value is 1415 mg Kg^{-1} with a standard deviation of 858 mg Kg^{-1}. Also in this case greater than 60 %.

Nitrate concentrations in iceberg lettuce samples ranged from 1210 to 2335 mg Kg^{-1}, with a mean concentration of 1813 mg Kg^{-1} and standard deviation of 19 %. In one sample, the concentration (2335 mg Kg^{-1}) is higher than the legal limits of 2000 mg Kg^{-1} for iceberg lettuce grown in open fields. In Table 3, the nitrate concentrations obtained by us are compared with those reported in the literature that refer to several samples from various locations and different analytical methods (Table 4).

As can be seen from the Table 3, the nitrate concentrations quantified by us on vegetables collected in Italy are in good agreement with those coming from other parts of the world and analyzed by different analytical methods.

The concentrations obtained by us are comparable to those present in the literature for the various categories. Only a sample of fresh spinach had a nitrate content higher than that established by Regulation (EU) 2023/915. Exceeding the regulatory limits results in the product's non-compliance with food use. In such cases, the competent authorities and the manufacturer must conduct a thorough analysis to identify the extent and geographical distribution of the non-compliant foods, the causes of the exceedances, and study possible ways to improve product quality.

Considering the quantities of the different vegetables consumed on average daily by the various categories of consumers, it can be deduced that on average the average ADI is between 63 % and 92 %.

Fortunately, according to some researchers (Ebrahimi et al., 2020),

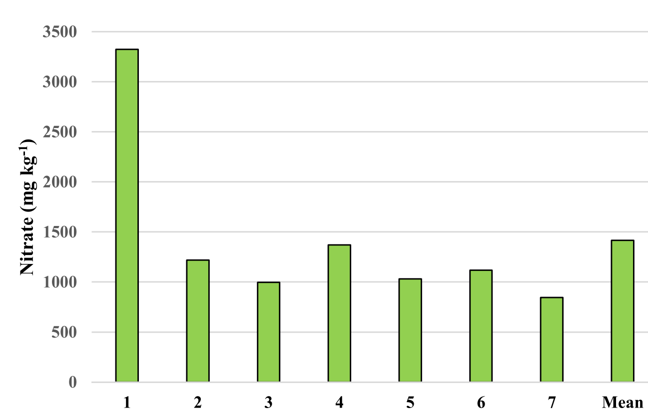

Fig. 10. Nitrate concentrations in different lettuce samples.

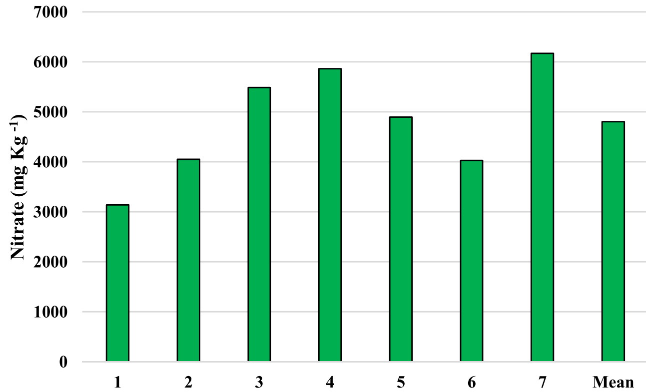

Fig. 11. Nitrate concentrations in different rocket samples.

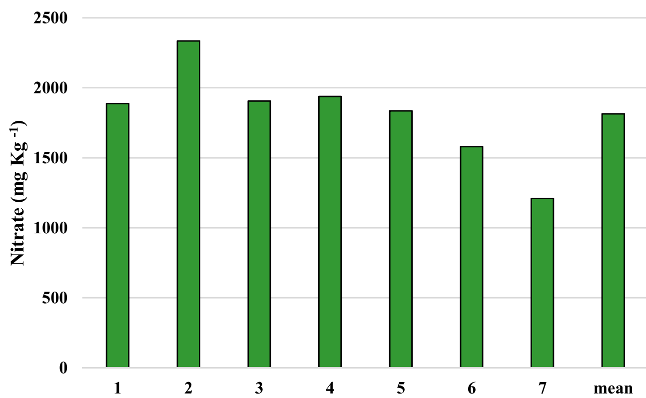

Fig. 12. Nitrate concentrations in different Iceberg samples.

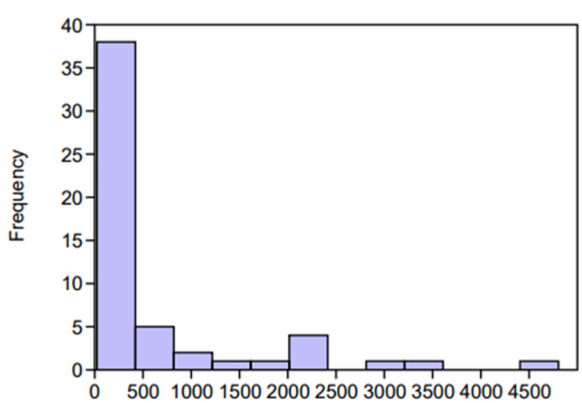

Fig. 13. Data set.

during cooking of vegetables, most of the nitrates present are released. Therefore, boiling food and not consuming the water for cooking decreases the nitrate content. Since controlling the concentration of nitrates during agriculture production is almost impossible, more attention should be paid to other methods to reduce their content that are mainly based on the methods of preparation, processing and cooking. For example, it has been reported that boiling can decrease the nitrate amount of green leafy vegetables by 47–56 %, while frying increases the content and flash freezing does not bring significant changes. Polish researchers (Smoczynski, 2004) studied the effect of their different traditional cooking practices on the nitrate amount of potatoes, establishing a reduction from 61 % to 98 % using different methods. It would be useful to quantify the concentrations of nitrates in ready-to-eat foods prepared in different ways (baked, grilled, etc.).

Table 3
Nitrate concentrations obtained by us compared with literature data.

Sample	Mean concentration from this paper (mg Kg^{-1})	Concentrations from literature (mg Kg^{-1})	Mean Concentrations from literature (mg Kg^{-1})	Reference
Lettuce	1415	1936	1527	(Ximenes et al., 2000)
		1297		(Prasad & Chetty, 2008)
		2728		(Chung et al., 2003)
		203		(Andac et al., 2011)
		1473		(De Martin & Restani, 2003)
Iceberg lettuce	1813	1074	819	(Petersen & Stoltze, 1999)
		355		(Ozdestan & Uren, 2010)
		999		Tamme et al., 2010)
		890		(Raczuk et al., 2014)
		777		(Ysart et al., 1999)
Rocket	4804	4474	4677	(Pardo-Marín et al., 2010)
		2597		(Santamaria, 2006)
		8150		(Tamme et al., 2010)
		4677		((EFSA). 2008)
		3488		(Temme et al., 2011)

Table 4
Statistical data.

Parameter	Value	Parameter	Value
N	54	Median	154
Min	23	25 %il	75.75
Max	4804	75 %il	653.75
Sum	33111	Skewness	2.41
Mean	613	Kurtosis	6.25
Std. error	133	Geom. mean	225
Variance	960105	Coeff. var	160
Stand. dev	979		

3.1. Statistical analyses

The nitrate concentration in 54 samples ranged from 23 to 4804 mg Kg^{-1}, underlining a heavily right-skewed distribution (mean ≈ 613 vs. median = 154; skewness ≈ 2.41). This is reflected in a wide interquartile range (75.75–653.75) and a standard deviation (979.8) that exceeds the mean, resulting in a coefficient of variation of ~160 %. These characteristics highlight the high variability in nitrate concentrations in the analysed samples. However, the Anova test performed on two groups (fresh vs. frozen) not underline significative differences ($F_{calculate}$ = 1.5642). However, the Anova test performed on two groups (fresh vs. frozen) not underline significative differences (F calculated = 1.5642 < F tabulated = 1.9550).On the other hand, the histogram in Fig. 12 clearly shows that the dataset is highly right-skewed: over one-third of all observations fall in the first one or two bins (roughly 0–500 μg Kg^{-1}), with the tallest bar around the 100–200 μg Kg^{-1} range. Frequencies then steadily decline as concentrations increase, forming a long, thin tail stretching towards the maximum value of around 4800 μg Kg^{-1}. There are only a handful of cases above 2000 μg Kg^{-1}, which highlights how a small number of very large measurements can drive the mean far above the median.

4. Health risk assessment

The World Health Organization and the Food and Agriculture Organization (WHO, 2004) recommend that individuals consume 400 g day^{-1} of vegetables and fruits to avoid chronic diseases. An acceptable daily intake (ADI) of nitrate of 3.7 mg Kg^{-1} day^{-1} were established by the WHO using data based on the risk stablished by EFSA, 2017 (EFSA, 2017). The consumption of vegetables food, consequentially, has been associated with a higher intake of nitrate amounts, in addition to other environmental pollutants (Santino Orecchio et al., 2022).

In this study, the health risks by the daily exposure to nitrate of vegetables, for adults and children, was estimated using Eqs. (1) and (2) introduced by USEPA (ASSESSment, 1989). Non-carcinogenic risks for nitrates in the vegetables samples were assessed by calculating chronic daily intake, hazard quotient (HQ), and target hazard quotient (THQ) using the following equations (Fallahzadeh et al., 2018; Mahdavi et al., 2022).

$$CDI \quad (mg\ Kg^{-1}\ day^{-1}) = \frac{C \times f \times IR \times EF \times ED}{BW \times AT} \tag{1}$$

$$HQ = \frac{CDI}{RfD} \tag{2}$$

C represents the nitrate concentration in vegetable food sample (mg Kg^{-1}), f is the conversion factor (g to Kg = 0.001), IR the mean daily vegetable consumption (400 g day^{-1}), EF the exposure frequency (350 days), ED the exposure duration (adults = 26 years, children = 6 years), BW_a is the average body weight for adults (80 Kg) (US-FDA, 2006) and for children (15 Kg) (Anyanwu and Adetunji, 2018; Mohammadpour et al., 2024), AT is the average life time days Fixed Non-carcinogenic = ED × 350.

The reference dose (RfD) for nitrate has been set at 1.60 mg $kg^{-1}day^{-1}$. In our case, the HQ is expressed as the ratio of the amount of exposure to nitrate over a specified period of time to the RfD for that substance derived from a similar period of exposure. If the estimated HQ is equal to or greater than 1, the exposure exceeds the RfD and exposed individuals may be at risk of adverse health effects, and vice versa.

HQ values are shown in Fig. 14 for two groups (children, and adults) exposed to nitrate through fruit and vegetables consumption. Considering all the samples analysed, for adults, the highest HQ values were found for some samples of broad-leaf foods: rocket (HQ=15), lettuce (HQ=10.4), beetroot (HQ=8.8), while for children HQ values ranged from 0.38 relating an apple sample to 80 quantified in a rocked sample.

Similarly, other researchers (Qasemi et al., 2024) for adults and children have evaluated HQ values higher than 1. In particular, for lettuce consumption the values are respectively 6.3 and 10.2, even though for children they estimated a consumption of only 154 g day^{-1} of vegetables.

According to an EFSA expert panel (EFSA, 2008), based on our data, it is possible to easily exceed the ADI by consuming approximately 50 g of lettuce per day, without taking into account other sources of nitrate exposure. They also note, however, that daily consumption of this amount of vegetable is highly unlikely in the long term, and therefore believe that exceeding the ADI on a single occasion does not pose a health concern. Josef Schlatter, Chair of EFSA's Scientific Panel, says that the positive effects of eating vegetables outweigh the risks posed by the nitrates they contain.

Furthermore, the CONTAM Panel emphasized that nitrate intake could be further reduced by certain measures, such as washing or peeling vegetables and/or cooking them, thus increasing the safety margin.

5. Conclusions

From the results obtained, it is clear that the optimized method for the potentiometric determination of nitrates, using an ion-selective electrode, was accurate, precise and selective even for the matrix taken into consideration, without a doubt, is much more complex than the aqueous ones. Interferences due to halides, in particular chlorides, always present in food samples, were eliminated. Furthermore, considering the quantities of material analysed (5–10 g) and the necessary dilutions, the quantification limits were satisfactory, although higher than those of more complex methods. The latter, for example chromatographic ones, require very expensive instruments both for purchase

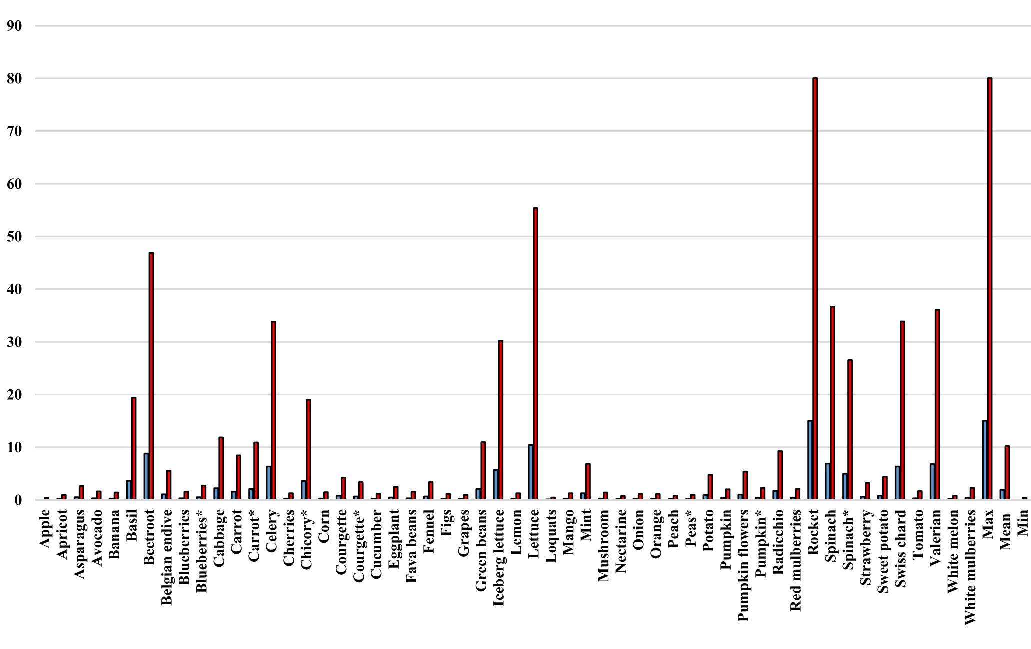

Fig. 14. HQ values relating to investigated vegetable samples.

and maintenance, indirect procedures, for example by reduction to nitrites are very laborious, furthermore, in all cases it is necessary to analyse solutions free of suspended substances, obtained through long and complex operations. Therefore, for the vegetable matrices examined in this study, the potentiometric method proved to be simple, rapid, economical and effective, having allowed the determination of even low concentrations of analyte such as those of fruit.

CRediT authorship contribution statement

Salvatore Barreca: Writing – original draft, Validation, Software, Data curation. **Giuseppe Arrabito:** Writing – original draft, Validation. **Giuseppe Di Benedetto:** Validation, Data curation. **Santino Orecchio:** Writing – original draft, Validation, Investigation, Data curation. **Silvia Orecchio:** Writing – original draft, Validation, Data curation. **Diana Amorello:** Writing – original draft, Validation.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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