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Harnessing nitrous oxide in post-harvest management of fresh horticultural produce

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ABSTRACT

High post-harvest losses in fresh horticultural produce and the increasing apprehensions among the consumers for harmful chemical residues have made it imperative for researchers to find safe, novel and natural techniques to achieve augmentation in shelf-life without having any detrimental influence on human health. Nitrous oxide, commonly known as "Laughing gas" is a naturally occurring colourless and non-flammable atmospheric gas. In the recent past, several researchers have documented that nitrous oxide gas inhibits ethylene production as well as action in freshly harvested fruits and vegetables. It also exhibits high potential in inhibiting fungal growth and decay, consequently reducing post-harvest losses due to diseases. Owing to its non-toxic nature, nitrous oxide can be potentially used to delay ripening and senescence of fresh horticultural produce during post-harvest storage and to assure food safety. In the present review, we have mainly focused on various effects of nitrous oxide on postharvest decay, ethylene biosynthesis and its action, respiration and other physico-chemical attributes of fruits and vegetables. Post-harvest application of nitrous oxide may open up various opportunities for its commercial use to prolong storage and marketability of fresh horticultural produce.

Key words: Decay, Ethylene, Fruit ripening, Nitrous oxide, Post-harvest

Fresh fruits and vegetables are highly perishable after harvest which causes a significant loss of the harvested produce. Synthetic pesticides and other chemicals are used indiscriminately to control the decay and post-harvest loss of the fresh horticultural produce. However, with the increasing concerns among consumers about health and harmful effects of pesticide residues on human health and environment lead the researchers to search for safe post-harvest technologies which can enhance the produce shelf-life and retain the inherent nutritive value of the food up to consumer end (Asrey *et al*. 2008). Several reports have been published about the harmful effects of synthetic chemicals on human health and environment (Ritter *et al*. 1995, Siddiqui and Dhua 2010). Recently, there has been a great interest on exploiting the potential benefit of some atmospheric gases for post-harvest management of fruit and vegetables (Spencer 1995). Nitrous oxide (N_2O) commonly known as 'laughing gas', is a naturally occurring atmospheric gas. In the atmosphere, the gas is principally produced by aerobic denitrifying bacteria present in soil (Firestone and Davidson 1989). However, its emission can be increased by addition of nitrite fertilizer in the soil (Shepherd *et al*. 1991). At room temperature, N_2O remains as inert and chemically neutral gas. Similar to carbon dioxide (CO_2), N_2O also has a linear structure (isostery) which confers similar physical properties like relative stability and high solubility to both the molecules (Leshem and Wills 1998, Benkeblia and Varoquaux 2003). Nitrous oxide is also classified as greenhouse gas with high global warming potential. Compared to carbon dioxide, N_2O has 298 times the ability to trap heat in the atmosphere on per molecule basis however; due to lower concentration in the atmosphere it contributes only 6% to total global warming (Williams *et al*. 1992). The effect of N_2O on post-harvest management of fresh fruits and vegetables has been investigated by few researchers. The available evidences suggest that it plays an important role in reducing disease incidence and enhances shelf-life by reducing respiration and ethylene production rates. Although, very limited work were done till now on the effect of N_2O on post-harvest management of horticultural produce, the present review attempts to sum up various effects of N_2O as reported by researchers (Table 1), its application and future prospects.

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Brief history of nitrous oxide

Nitrous oxide was first discovered in 1772 by Joseph

Table 1 Summary of some effects of nitrous oxide on harvested fruit and vegetables

Commodity	Effect of nitrous oxide	References
Tomato, avocado	Delay climacteric ethylene rise, inhibit ethylene production	Gouble *et al*. (1995)
Banana	Decrease respiration and ethylene production rate, do not influence physico-chemical quality attributes (soluble solids, pulp and peel firmness, colour, starch content, titratable acidity and pH), increase weight loss	Palomer *et al*. (2005)
Onion	Reduce respiration rate, fungal rot; slightly increase soluble sugars and organic acid accumulation; no effect on sprouting	Benkeblia and Varoquaux (2003)
Strawberry	Reduce respiration rate and fungal decay, no effect on quality	Qadir *et al*. (2000); Qadir and Hashinaga (2001a)
Apple, mandarin, persimmon, guava, tomato,	Inhibit postharvest decay in artificially inoculated fruit	Qadir and Hashinaga (2001a)
Longkong	Reduce pericarp browning	Lichanporn and Techavuthiporn (2013)
Fresh-cut pineapple	Inhibit respiration and ethylene production, delay softening, microbial growth	Rocculi *et al*. (2009)
Fresh-cut kiwifruit	Reduce changes in texture, colour, soluble solids content and weight loss	Rocculi *et al*. (2005)

Priestley. Later in the year 1800, Humphrey Davy of the Pneumatic Institute in Bristol, England observed its physiological properties and coined the term "laughing gas". However, until 1840, its primary use was for the recreational purpose. In 1844, Gardner Quincy Colton put on a demonstration on N_2O in Hartford where, Horace Wells noted the analgesic property of the gas and continued its use as an anaesthetic agent. After 150 years, he was recognized as the "Discoverer of anesthesia". However, after death of Horace Wells in 1848, it was Colton who introduced the use of N_2O more broadly at Colton Dental Association clinics which was founded by him in New Haven and New York City. Since then, it is still the agent of choice for anaesthesia for dentistry and a standard analgesic used in pre-hospital care and obstetrics (Smith and Hirsch 1991).

Physical properties of nitrous oxide

Nitrous oxide is a colourless non-flammable gas with a slight sweetish odour and taste. It's molecular weight is 44, specific gravity 1.53 and boiling point –89°C. The gas is synthesized in the laboratory by heating ammonium nitrate crystals to about 240°C temperature, chemical scrubbing followed by compressed (50 atmosphere) to a liquid form in blue coloured pressurized tanks. In the pressurized tanks, N_2O exist in both liquid and gaseous forms. The unique property of N_2O is that in the pressurized tanks, with the release of gas its pressure does not decrease until 75 – 80% consumption. This is due to the fact that up to that amount the gas remains in liquid form whereas in other compressed gases, it exists only in gaseous form which shows continuous decrease in pressure with the use.

Nitrous oxide in post-harvest management of fruit and vegetables

Post-harvest decay control: Fresh fruits and vegetables are highly susceptible to a variety of pathogenic attack once they are harvested from the plant. To minimize the incidence of post-harvest decay during storage, several synthetic fungicides have been commercially used for many years. However, increasing consumers concern over pesticide residue on fresh horticultural crops, their harmful effects on human health and environment results need to explore new safe alternatives to replace the use of chemicals. Moreover, indiscriminate use of synthetic fungicides may also result in development of fungicide-resistant strains.

Studies have revealed that nitrous oxide can be used as a potent alternative to control post-harvest decay of fresh fruit and vegetables. Enfors and Molin (1977) reported the effectiveness of N_2O against chemically induced germination and growth of *Bacillus* spores. Similarly, fungistatic property of N_2O at low pressure has also been postulated against *Escherichia coli*, *Saccharomyces cerevisiae* and *Tetrahymena thermophila* (Thom and Marquis 1984). Qadir and Hashinaga (2001a) tested the efficacy of nitrous oxide in inhibiting the post-harvest decay against common fruit-infecting fungi. Artificial inoculations were performed in apples cv. Fuji with *Alternaria alternata* and *Penicillium expansum*; strawberries cv. Toyonoka with *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *fragariae* and *Rhizopus stolonifer*; satsuma mandarin with *Geotrichum candidum*; persimmon cv. Fuyu with *Colletotrichum acutatum*; seedling guava with *Rhizopus stolonifer* and tomato cv. Momotaro with *F. oxysporum* f. sp. *lycopersici*. Appearance of disease and lesion growth rate in these artificially inoculated fruit was significantly delayed by exposure of fruit to 80% N_2O + 20% oxygen (O_2) atmosphere, the response being dose and time-dependent. However, it was not proved similar effectiveness against all the fungi. During evaluation the *in vitro* fungistatic or fungicidal property of N_2O against twelve important post-harvest fungi exposed to 10 – 80kPa N_2O and 20kPa O_2, *Alternaria alternata*, *Fusarium oxysporum* f.sp. *fragariae*, *F. oxysporum* f. sp. *Licopersici* and *Geotrichum candidum* showed lower inhibition of fungal colony than others (Qadir and Hashinaga 2001b). On the basis of effectiveness, they divided the fungus into N_2O high, medium and low-sensitive groups. In another study, treatment of onion bulbs with N_2O at a concentration of 50, 80 and 100 kPa for 5, 10 and 18 days significantly reduced the incidence of fungal rots when compared to bulbs stored at air and nitrogen atmosphere (Benkeblia and Varoquaux 2003).

Post-harvest decay control by N_2O is due to fungistatic property of the gas. It is postulated that *in vitro* antifungal or antibacterial property of N_2O is due to interference in methionine pathway (Frontiera *et al*. 1994) and inhibition of ethylene action (Gouble *et al*. 1995), which is essential for fungal growth (Kepczynska 1989, 1993). This was confirmed by addition of AOA (amino oxyacetic acid), an inhibitor of ethylene biosynthesis to the culture of *Botrytis cinerea*, showed reduction in mycelium growth due to absence of ethylene in the medium (Qadir and Hashinaga 2001b). However, this mechanism is not true in case of *in vivo* fungal growth inhibition. This is due to the fact that methionine is readily available from the host tissue and the infected tissue produces abundant ethylene (Abeles *et al*. 1992). Thus in this situation, N_2O might have worked indirectly by increasing the defence response and resistance in the host tissue (Qadir and Hashinaga 2001a). Moreover, higher CO_2 concentration is also reported to suppress fungal growth (El-Goorani and Sommer 1981). Thus, structural similarity of CO_2 with N_2O also explains the mechanism in inhibiting fungal growth.

Nitrous oxide delays fruit ripening

Effect on ethylene biosynthesis: Ethylene, the ripening hormone plays a major role in ripening and senescence of fruits. Post-harvest treatment with N_2O effectively decreases ethylene biosynthesis and its action in fresh horticultural produce (Leshem and Wills 1998). Gouble *et al*. (1995) reported that continuous gas treatment of tomato and avocado fruit with 80% N_2O + 20% oxygen significantly inhibited the ethylene production at both pre-climacteric and climacteric stages. In these fruit, treatment with N_2O extended the lag phase of ethylene with a delay in climacteric peak by about 8 and 4 days, respectively, than control. However, its effect on reducing ethylene evolution after the onset of climacteric showed mixed response. In tomato, N_2O inhibited the autocatalytic production of ethylene at later climacteric stages (Gouble *et al*. 1995) however, it was not able to reduce the rate of ethylene rise or delay the ripening process in avocado (Gouble *et al*. 1995) and banana (Palomer *et al*. 2005) once it is initiated. The treatment of tomato fruit with exogenous ethylene followed by storage in N_2O reversed the induced autocatalysis in tomato and showed drastic reduction in ethylene evolution. N_2O also proved its effectiveness in delaying the upsurge of ethylene in tomato during senescence by inhibiting ACC synthase synthesis and activity, thus delaying the ACC accumulation and its conversion into ethylene (Gouble *et al*. 1995). Moreover, research findings have revealed that the effect of N_2O in inhibiting ethylene evolution is highly dose and time-dependant. In banana, N_2O treatment (80% N_2O+ 20% O_2) of fruit for 48 h was not effective in retarding fruit ripening however, its exposure for 5 or 10 days delayed the onset of ripening (Palomer *et al*. 2005). Similar results were found in tomato and avocado fruit (Gouble *et al*. 1995). These authors have demonstrated that the solubility of N_2O in fruit cells is about 77% approaching the value for CO_2, however, its absorption in fruit tissue is entirely reversible (Fath *et al*. 1990, Gouble *et al*. 1995).

The induction of ethylene biosynthesis in fruit is a feed-forward biochemical mechanism, often referred to as autocatalysis (Peacock 1972). It has been proposed that two systems regulate the ethylene biosynthesis in plants, System-I and System-II (McMurchie *et al*. 1972). In immature developing fruit, non-autocatalytic System-I is responsible for inducing ethylene biosynthesis. However, during onset of ripening rapid upsurge of ethylene takes place in climacteric fruit due to initiation of an autocatalytic System II. At this stage, System-I ethylene is thought to bind with the receptors, causing the upregulation of ACC (1-aminocyclopropane-1-carboxylic acid) synthase and ACC oxidase (Barry *et al*. 2000). This autocatalytic biosynthesis of ethylene requires the constant presence of the ethylene (Peiser 1989). Exogenous application of N_2O is proposed to inhibit ethylene biosynthesis in fruit by two way mechanisms (Fig 1): (i) Formation of N_2O-ethylene complex in System-I, by binding N_2O with ethylene. As a result, the produced ethylene cannot act on its receptor until the concentration of ethylene reaches a critical threshold level in System-I; or (ii) Inhibit synthesis or activity of ACC synthase and ACC oxidase in System-II (Gouble *et al*. 1995, Bemish *et al*. 1996). The presence of oxygen is a prerequisite for binding of ethylene to its receptor. Lower oxygen tension and elevated CO_2 level acts as ethylene action inhibitor by displacing ethylene at the receptor site (Burg and Burg 1967) and affecting the activity of ACC oxidase enzyme (Poneleit and Dilley 1993, Yip *et al*. 1988). This blocks the upregulation of ethylene biosynthesis. Gouble *et al*. (1995) postulated that due to biophysical similarity of

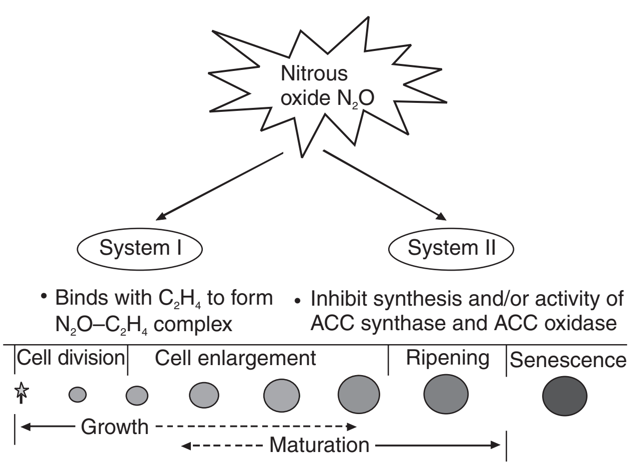

Fig 1 Schematic overview of mechanisms by which N_2O inhibits ethylene production.

N_2O with CO_2, the mode of action of both the molecules is similar. However, studies on strawberry in extending shelf-life of fruit through N_2O and CO_2 (Qadir *et al*. 2000) suggests that the inhibition of ripening by N_2O might be through System-II ethylene-independent mechanism. This is due to the fact that owing to non-climacteric in nature, ripening of strawberry does not depend on climacteric rise of ethylene. It supports that N_2O-ethylene complex formed in System-I temporarily inhibit the ethylene action on its receptor and delay the ripening process. In another study on banana fruit, Palomer *et al*. (2005) reported that N_2O could not able to decrease the ACC content and ethylene production in System-II. These authors suggested that N_2O might have competitively inhibited ACC oxidase activity until the produced ACC reached a critical threshold level. When it exceeds the threshold limit it initiated the onset of ripening, which is an irreversible process.

Effect on respiration rate: Nitrous oxide has been reported to slow down the rate of respiration in harvested fruit and vegetables. In onion bulbs, about 50% reduction in respiration rate was recorded after 5 days of treatment with N_2O, due to presence of anoxic atmosphere (Benkeblia and Varoquaux 2003). Sowa *et al*. (1987) reported that the reduction in respiration rate by N_2O is caused by a reversible partial inhibition of oxygen consumption by mitochondria. It is postulated that N_2O binds with lipids and proteins such as cytochrome *c* oxidase in mitochondria and thus lowers the respiration rate (Gouble *et al*. 1995, Day 1996). Sowa and Towill (1991) found lower cytochrome *c* oxidase activity in mitochondria isolated from seeds, leaves or cellular suspensions. Similarly, beneficial effect of N_2O storage in reducing respiration rate is also reported in banana (Palomer *et al*. 2005) and fresh cut pineapple (Rocculi *et al*. 2009). Moreover, respiration and ethylene production rates in fresh horticultural produce are an interlinked process. An increase in production and activity of ethylene also increases the respiration rate of the crop. Thus, another mechanism of N_2O in reducing respiration rate is due to decrease in ethylene production and action along with the negative effects on ACC synthase and ACC oxidase activity.

Effect on physico-chemical quality: Ripening and senescence of fruit is accompanied with several changes in the physico-chemical quality attributes such as changes in colour, increase in sugars content, reduction in firmness, titratable acidity, production of flavour volatile compounds *etc*. (Wills *et al*. 1968). Similarly in vegetables, several compositional changes take place with its maturity and senescence. Treatment of banana fruit with N_2O at a concentration above 40% along with oxygen (8 or 12%), significantly delayed fruit ripening, due to its anti-ethylene activity (Palomer *et al*. 2005). However, it did not influence the quality parameters like colour, firmness, starch and soluble solids content, acidity and pH with regard to the control. Most notably, the weight loss of these fruit exposed to continuous treatment with 40 – 60% N_2O found higher than control which is correlated with the length of pre-climacteric lag phase while, it was not observed under short term treatment for less than 10 days. Similarly, no effect of N_2O treatment on physico-chemical quality of strawberry fruit was reported by Qadir *et al*. (2000). Conversely in case of avocado, an increase in organoleptic quality with N_2O treatment was reported by Gouble *et al*. (1995). In case of onion, Benkeblia and Varoquaux (2003) reported a slight increase in soluble sugars content and accumulation of organic acids (citric acid, succinic acid, fumaric acid, malic acid, oxalic acid) following treatment of bulbs (*Allium cepa* cv. Rouge Amposta) with N_2O. The authors proposed that the effect of stress on cellular catabolism during storage in N_2O-atmosphere is similar to that induced by anoxia. However, it was reduced and being diverted to the fermentative pathway after treatment with N_2O, as there was no high production of CO_2. No effect of N_2O treatment on sprouting of onion bulbs was recorded (Benkeblia and Varoquaux 2003, Benkeblia *et al*. 2001). Exposure of longkong fruit (*Aglaiado okkoo* Griff.) to 90% N_2O for 3 h maintained higher phenolic compound and delayed pericarp browning by lowering activities of phenylalanine ammonia lyase, polyphenol oxidase and peroxidase enzymes (Lichanporn and Techavuthiporn 2013, Lichanporn *et al*. 2013).

Nitrous oxide maintains quality in minimally processed produce

Minimally processed products are fresh-cut fruits and vegetables which maintain their quality similar to fresh products (Alzamora *et al*. 2000). The major problem in maintenance of quality in minimally processed products is their high perishability. This is because during peeling and cutting of produce, the internal tissues are exposed which causes loss of cellular compartmentation, mixing of enzymes with their substrates and it's associated browning. Moreover, due to physical damage, it accelerates the respiration and ethylene production rate, which ultimately affects the shelf-life and quality of produce (Mazliak 1983, Watada *et al*. 1990). The potential impact of N_2O as a packaging gas for fresh-cut fruits were reported in pineapple and kiwifruit. Packaging of kiwifruit slices in modified atmosphere, containing 90% N_2O, 5% O_2 and 5% CO_2 at 4°C was highly effective in preserving quality of minimally processed kiwifruit by reducing changes in texture, colour, soluble solids content and weight loss (Rocculi *et al*. 2005). The authors proposed that N_2O lowers the activity of enzymes responsible for softening (pectinesterase, polygalacturonase and β-galactosidase) and colour change (chlorophyllase) indirectly, by reducing the respiration and ethylene production rates. Owing to higher solubility of N_2O (0.665g L^{-1}) in water, it readily dissolves in the aqueous layer of the cut fruit cells. It inactivates the chemically-active sites of the enzymes and reduces the level of dissolved oxygen, which is highly essential for oxidative enzymes to catalyse metabolic reactions in the fruit (Rocculi *et al*. 2005). A combined application of 1-MCP (1-methylcyclopropene) treatment and modified atmosphere packaging of fresh-cut pineapple in N_2O-

enriched atmosphere (86.13kPa N_2O, 10.13kPa O_2 and 5.07kPa CO_2) decreased respiration and ethylene production (Rocculi *et al*. 2009). The lag phase of microbial growth is extended by 3 – 4 days by the treatment.

Other important applications of nitrous oxide

Applications in food industry: Nitrous oxide is widely used as a propellant gas of food aerosol products. It is most widely used in whipped cream canisters because of higher foaming stability than CO_2 (Nakai 2005). In addition, the taste of N_2O-containing whipped cream is similar to air-whipped cream. The gas is also used as an inert gas to displace oxygen in packaging of potato chips and other snack foods. In dairy products, the gas is used to provide foaming and inhibit rancidity.

Applications in medical science: Nitrous oxide is the oldest and only inorganic anaesthetic agent still used in the medical science. It is principally used in dentistry as an analgesic and mild sedative (Neidle and Yagiela 1989). The gas is usually administered through a mask along with 20% oxygen to prevent oxygen deprivation. The gas is much less toxic than other alternative anaesthetic like chloroform. In paediatrics, N_2O is used to help aid behaviour management in those children in whom more conventional measures have proven unsuccessful.

Safety concerns

The safety of nitrous oxide to human being should not be questioned since the gas is approved for use as food additive (also known as E942) by CODEX Alimentarius Commission. Following extensive review, U.S. Food and Drug Administration (USFDA) has also affirmed N_2O as generally recognized as safe (GRAS) as a direct human food ingredient (21CFR184.1545). It is most commonly used as a propellant and foaming agent of aerosoltype whipped cream and its food simulants. Presently, the gas is widely used in over 30 countries including USA and European countries as a food additive (Nakai 2005).

Future prospects

Although, N_2O is widely used in medical science since centuries however, its use in post-harvest quality management of horticultural crops is at infant stage. A considerable work is still required to exploit its potential in reducing post-harvest loss of fresh produce. The mode of action of N_2O in inhibiting ethylene production and action is partly elucidated. Further detailed experiment is required to understand the mechanisms of N_2O action on ethylene in both climacteric and non-climacteric crops. The response of fruit and vegetables to exogenous N_2O treatment is heterogeneous as its efficacy varies with species, physiological stage of the crop and experimental conditions such as dose and duration of exposure. More information is required on the number of fruit and vegetables that may respond to N_2O as well as its best and safe concentration for each crop cultivar. Investigation should also carry out on its defence mechanism against pathogen infection. It may be possible that N_2O induce the release of signalling molecules like methyl jasmonate, nitric oxide or salicylic acid at the pathogen attack site. Future work should also focus at the molecular details of the underlying mechanisms of anti-ethylene and antifungal property of N_2O. Since, synergistic effect of N_2O with other post-harvest treatments such as 1-MCP has been observed thus, use of N_2O in combination with other post-harvest treatments may give better result in minimizing post-harvest losses of fruit and vegetables.

Nitrous oxide, a non-toxic atmospheric gas exhibits immense potential in post-harvest quality management of fresh fruit and vegetables. Research has revealed that N_2O treatment inhibits ethylene production and action at both pre-climacteric and climacteric stages of fruit development, thereby delays ripening and senescence of fresh produce. Continuous exposure of fruit and vegetables to N_2O may be a useful and promising measure to minimize incidence of postharvest decay during storage. N_2O can be potentially used as an alternative to synthetic harmful chemicals in post-harvest technology of fresh horticultural produce to assure food safety to the consumers.

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