Novel Applications of Gamma Irradiation on Fruit Processing

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Abstract



Purpose of Review Consumers increasingly demand for fruit products as a source of health-promoting compounds. However, fruits have a limited shelf life and their processing with thermal technologies significantly reduces their bioactive compounds. Instead, the application of a non-thermal technology like gamma irradiation could overcome this limitation. This review summarizes the latest research on the application of gamma irradiation on fruit processing.

Recent Findings Evidence shows that gamma irradiation produces positive effects on fruit products: fungal and microbial (spoilage and pathogenic) disinfection and decontamination, deparasitization, and delayed ripening and senescence, leading to a longer shelf life without affecting their quality. Besides, this technology is also being tested as a stress elicitor to induce physiological defense mechanisms in plant fruit tissues.

Summary The gamma irradiation process represents an opportunity to expand the export of value-added products over long distances, given its high efficiency to extend the shelf life of fruit products. Therefore, this paper provides a review of the currently available applications and effects of gamma irradiation in fruit processing. Considering the high cost associated to the refrigerated storage and transport of fruits, ionization is expected to give birth to a new era in the international trade of foods.

Keywords Food preservation \cdot Ionizing radiation \cdot Fruit products \cdot Phytosanitary control \cdot Pathogen elimination \cdot Stress elicitor

Introduction

Gamma irradiation (GI) is a completely safe physical method of food preservation, applied in the form of ionizing radiation. It is also known as "cold sterilization" because of its capacity to inactivate microorganisms that may grow during the storage of food without any heat transfer, which makes it a non-thermal preservation technology [1].

In practice, this technology consists in exposing food products to the energy emitted by a radiation source for a certain period of time, so that the product absorbs a

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controlled amount of energy per unit of mass. In an industrial irradiation plant, the absorbed dose is controlled by a single parameter, the exposure time. Thus, with only one variable to control, the process has a high reliability and repeatability. One of the main advantages is that this technology does not generate industrial effluents. The products to be treated can be processed in their final packaging and consumed right after treatment without the need of quarantine [2, 3].

GI is used to achieve the following objectives: consumer health protection, phytosanitary control, extension of shelf life, and quality preservation of the final product. In terms of its proven applications, GI produces the following positive effects on fruit products: fungal and microbial (both spoilage and pathogenic) disinfection and decontamination, deparasitization, sprout inhibition, delayed ripening, and senescence, all these leading to a longer shelf life and eventually, the sterilization of foods [4].

GI proved effective to prevent the transmission of pathogenic diseases in humans. In addition, foods irradiated at doses considered as safe did not produce any teratogenic, mutagenic, or carcinogenic modifications, nor any

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significant nutritional change [5]. GI also represents a promising alternative to fumigation, making it possible to reduce or eliminate the use of chemical agents such as methyl bromide, a substance banned by the Montreal Protocol [6]. On the other hand, GI can be considered as an environmentally sustainable process, able to render healthy fruit products with extended shelf life, and no refrigeration requirement. Moreover, a significant amount of energy can be saved compared to other technologies, since no heat is used during the process.

Given that this technology is able to reduce food losses and waste along the entire supply chain, it is expected to represent an opportunity to increase the exportation of valueadded products over long distances, given its high efficiency in extending the shelf life of fruit products. All these positive aspects considered, this paper provides a review of the currently available applications and effects of GI in fruit processing. The novelty of the work is to describe recent laboratory-scale research indicating that GI can be used to preserve functional bioactive compounds beneficial to human health, and as a stress elicitor to induce physiological defense mechanisms in plant fruit tissues.

Basic Principles of GI and Description of Its Effect on Food Components

Gamma irradiation is a food processing technology that involves exposing products to ionizing radiation in the form of gamma rays, emitted by radioactive isotopes such as cobalt-60 or cesium-137. Gamma rays are generated from photons, which are bundles of electromagnetic waves moving at the speed of light, located in the electromagnetic radiation spectrum, in the high-frequency and high-energy region. GI has an undulatory nature, lacking mass and charge, similar to ordinary light but with a shorter wavelength [7].

The technology exerts both direct and indirect physical, chemical, and biological effects on matter (Table 1). Radiation interacts with organic and inorganic compounds, generating excitations and electronic ionizations, the latter being the predominant effect. In this way, radiation can remove electrons from atoms or molecules, which will therefore become charged (ionized). These chemical species are usually unstable, and can rapidly react with their neighboring molecules, exciting and/or recombining them or producing fragmentations [8]. The chemical changes generated can affect the components of food and the cells of the organisms that may be present, with biological consequences at the level of cellular activity and functionality.

Water Radiolysis

GI can cause a wide range of chemical changes on the irradiated matter through direct and indirect effects. In terms of direct effects, radiation can act directly on food nutrients, ionizing them and breaking down molecules, giving rise, as in the case of water, to radicals that can combine each other differently from their original form. The main compounds that can undergo significant changes are proteins (including enzymes), carbohydrates, lipids, and vitamins.

Regarding the indirect effects, water plays a relevant role, since, in addition to its capacity to generate ions and free radicals — reactive oxygen species (ROS) — through ionization and radiolysis processes, it constitutes on average 80% of cellular matter (in fruit products it can attain almost 90% of its composition). In this way, radiation generates unstable radicals and reactive molecules with very short half-life (nanoseconds) [9]. However, this time frame is sufficient to combine with other components through oxidation, addition, and reduction reactions, initiating a series of competitive reactions as well as giving rise to new compounds by recombination of radicals.

Another factor influencing the effect of radiation is the potential presence of oxygen during irradiation, since the reducing agents formed in the radiolysis of water can react with this compound, leading to the formation of hydrogen peroxide. Therefore, the formation of this highly reactive compound in irradiated systems depends on the oxygen concentration. In this aspect, packaging in modified atmospheres can be useful to limit this process [1, 10].

Effect of GI on Microorganisms

GI affects microorganisms (bacteria, yeasts, and molds) by causing damage to the genetic material of the cells and interfering with the biological processes necessary for their

Table 1 Physical, chemical, and biological processes involved during the interaction of ionizing radiation on organic and inorganic matter

Physical process	 Involves the interaction of radiation with matter, producing excitation and ionization in organic and inorganic compounds Stability, 10⁻¹⁶-10⁻¹⁴ s
Chemical process	 The molecular species formed in the physical process react in a chain reaction with neighboring molecules. Their extent depends on whether the molecular species can diffuse into the medium Stability, 10⁻¹²-10⁻⁷ s
Biological process	• Involves chemical changes produced in cellular components, which may have consequences on cellular activity and func- tion

development [11, 12]. GI must be applied to foods in an intensity range able to inactivate microorganisms without affecting the quality, safety, and functionality of the food components.

Radiation interacts with genetic material and other cellular elements such as proteins and membranes, which are essential for the survival of organisms. Hydrogen bridges of the inter-catenary bonds of deoxyribonucleic (DNA) and ribonucleic acids (RNA) are particularly sensitive to this treatment, so that the damage can prevent cell reproduction or even cause the death of microorganisms [13].

On the other hand, radiation interacts with other atoms and molecules in the cell, including water, giving rise to complex reactions, some of them generating ROS, which can damage nucleic acids. This effect is important in vegetative microorganisms, whose cytoplasm contains about 80% water [14]. Although irradiation can also damage other structures such as cell membranes and inactivate enzymes, leading to metabolic alteration, the main target of this type of treatment is DNA [15].

Effect of GI on Proteins

GI can modify the secondary, tertiary, and quaternary structure of proteins, leading to changes in chain spatial arrangement and 3D configuration. Weak physical bonds such as hydrogen bridges or electrostatic bonds can be broken, bringing about protein aggregation and denaturation and, consequently, loss of protein functionality in aspects such as water holding capacity, rheological behavior, solubility, electrophoretic behavior, enzymatic activity, and immunological reactivity [10].

Enzymes are considerably more resistant to inactivation than microorganisms. In general, complete inactivation of enzymes requires a dose 5 to 10 times higher than that required for the destruction of microorganisms [16]. Despite this resistance, it is possible to reduce at low doses the enzyme activity to inhibit spoilage processes such as enzymatic browning, therefore extending the shelf life of fruit products. Besides, enzymes in solution can be also affected by the indirect effects of free radicals formed in the solvent.

Effect of GI on Carbohydrates

Because of their crystalline character, carbohydrates are sensitive to radiation, whose effect will depend on the dose applied: at low doses (up to 1.0 kGy), no breakdown of monomers such as glucose, fructose, and galactose will be provoked [17], while at medium and high doses, products such as H_2 , CO_2 , aldehydes, ketones, acids, and other carbohydrates can be generated [18].

In the presence of water, the direct and indirect action of radiation can cause oxidative degradation. The indirect action can provoke the attack of carbohydrates mainly by -OH radicals. These radicals would predominantly react with the hydrogen of the C-H bonds, forming H_2O , while in the presence of oxygen, secondary reactions can be also evidenced.

In the case of oligosaccharides, different monosaccharides and products similar to those obtained by irradiation of simple sugars can be formed. High molecular weight polysaccharides will also degrade due to the cleavage of the glycosidic linkage, forming smaller carbohydrates such as glucose or maltose [10]. Irradiation of fruit products may result in softening and loss of texture because of the breakdown of cell wall materials such as pectins and celluloses [18]. However, this softening can be beneficial, for example, by increasing juice extraction yield, and/or decreasing drying and cooking times in dehydrated products.

Effect of GI on Vitamins

The stability of vitamins to radiation is important when analyzing the nutritional aspects. Some vitamins are considered susceptible to ionizing radiation [19]. Although in complex media such as food matrices, the effect of radiation can be reduced. The typical doses and irradiation conditions used in food can largely alter vitamin stability [20].

The wide variety of chemical structures of vitamins determines that the effect of radiation also presents a great variability, depending on each vitamin. Among the water-soluble vitamins, vitamin B1 (thiamine) is the most susceptible to radiation, while vitamin C or ascorbic acid is also unstable to radiation, producing, among other products, dehydroascorbic acid [21]. However, these vitamins in fruit products are quite stable to irradiation at low doses [22]. Other watersoluble vitamins prone to radiation are vitamins B2 (riboflavin) and B12 (cyanocobalamin) and biotin. Among the fatsoluble vitamins, vitamin E is the most radiosensitive, while vitamin D is the most resistant. Vitamin A is also relatively susceptible because the induced cis–trans isomerization can decrease its activity.

Application of GI on Fruit Processing

Among the various potential uses of irradiation in food technology, the most promising applications are the decontamination, disinfestation, and quality preservation of food products to extend their shelf life [7] (Fig. 1). These applications are detailed below (Table 2). Fig. 1 Mechanisms of action of gamma radiation for processing/ enhancing the shelf life of fruits



Disinfection and Preservation

GI is effective in disinfecting fruits by reducing their pathogenic load, extending their shelf life. For instance, in table grapes, irradiation proved effective to reduce the levels of *Salmonella* spp. and *Escherichia coli* by over 90%, significantly enhancing the microbiological safety of the produce [24]. In strawberries, irradiation was able to eliminate pathogenic bacteria like *Escherichia coli* and *Listeria monocytogenes*, enhancing the safety of the produce [23]. This is crucial to minimize the risk of foodborne diseases. Other studies reported that an optimized radiation dose of 0.5 kGy inhibits in Ponkan fruit the growth of *Penicillium digitatum*, slowing down the rate of fruit decay [25•].

Insect and Pest Control

GI is a valuable tool for controlling insects and pests in fruit products. In fruits like oranges, irradiation can effectively eliminate larvae and eggs of fruit flies such as *Ceratitis capitata*, without causing damage to the tissues [27]. This preserves fruit quality and prevents pest proliferation. In papayas, the technology has been employed to prevent the proliferation of fruit flies such as *Bactrocera dorsalis*, preventing damage with suitable fruit quality [26].

Delay of Ripening

GI is applied to delay the ripening process in fruits like bananas. The irradiation of green bananas successfully inhibited ethylene production and the consequent formation of volatile compounds, delaying the ripening process. This allows for a longer storage and transportation period [29]. By irradiating mangoes, the production of ethylene and enzymes responsible for softening was reduced, extending the shelf life and maintaining fruit quality [28].

Reduction of Postharvest Losses

GI contributes to reducing postharvest losses in fruits by extending their shelf life [32]. In mangoes, irradiation can decrease the rate of weight loss and maintain the firmness, the color, and the sugar content of the product during storage [31]. This is essential for maintaining product quality and marketability. In table grapes, irradiation prevented the softening and shriveling of the fruit during storage, minimizing economic losses [30].

Pathogen Elimination

GI is an effective technique to eliminate pathogens in fruit products. In stone fruit such as peaches and plums,

Table 2 Applications of gamma irradiation in fruit products

Application	Fruit	Effects	Irradiation dose (kGy)	Reference
Disinfection and preservation	Strawberry	Elimination of <i>Escherichia coli</i> and <i>Listeria mono-</i> <i>cytogenes</i> (4 log CFU/g reductions)	1.0	[23]
	Table grape	Reduction of <i>Salmonella</i> spp. and <i>Escherichia coli</i> (90% reduction)	1.0	[24]
	Ponkan fruit	Inhibition of <i>Penicillium digitatum</i> (inhibition rate of 40%)	0.5	[25•]
Insect and pest control	Papaya	Fruit fly control Bactrocera dorsalis (sterility)	0.15	[26]
	Orange	Elimination of larvae and eggs of fruit flies <i>Cerati-</i> <i>tis capitata</i> (sterility)	0.15	[27]
Delay of ripening	Mango	Delay of ripening by inhibition of ethylene (9%) and ripening enzymes (polyphenol oxidase and peroxidase)	0.25	[28]
	Banana	Inhibition of ethylene production (23%), and delay of the formation of volatile compounds responsi- ble for ripening	1.0	[29]
Reduction of postharvest losses	Grape	Maintenance of firmness, reduction in wrinkles and softening (20%)	1.0	[30]
	Mango	Decrease in the weight loss rate (12%) and mainte- nance of firmness, color, and sugar content	1.0	[31]
	Mandarin	Decrease in weight loss (10%) and disease inci- dence (9%) as well as inhibition of the increase in soluble solid content and retention of higher titratable acidity (TA ratio 8.2%) after 90 days of storage	1.0	[32]
Pathogen elimination	Blueberry	Reduction of <i>Salmonella</i> spp. (5 log CFU/g reductions)	1.5	[33]
	Stone fruit	Elimination of <i>Escherichia coli</i> and <i>Salmonella</i> spp. (4 and 4.5 log CFU/g reductions, respectively)	1.5	[34]
	Apple	Inactivation of <i>Escherichia coli O157:H7</i> (3 log CFU/g reductions)	1.0	[35 ••]
Improved quality in minimally processed fruit products	Blueberry	Reduction of the fruit rot rate (3.35%) , maintaining the fruit firmness (1.08 N) and color, and reduction of the loss of nutrients during storage $(35 \text{ days}, 0 \text{ °C})$	2.5	[36]
	Peach	Changes on color, homogeneity, peach aroma, total flavor intensity, peach flavor, sweetness, and juiciness, increasing their intensities. Increase in brightness, total aroma intensity, peach aroma, and flavor and texture descriptors Retention of malic acid and increase in sucrose concentrations (20%)	1.0	[37]
	Blueberry	Improved shelf life of the different blueberry varie- ties. Maintenance of firmness, color, total soluble solids, and acidity. Increase in total phenols (23.4%) and anthocyanins (14.8%) during storage (7 days, 1 °C)	0.15	[38]
Stress elicitor treatment	Papaya	Induction of the initiation of ripening (product of cell wall enzyme synthesis, mainly PME) and rapidly improvement of the fruit's commercial firmness	0.5	[39]
Induction of the physiological mechanism of defense in fruit products	Peach	Overexpression of HSP (in particular, small HSP between 15 and 40 kDa). Promotion of the physiological and biochemical defense mechanisms of the fruit	0.1–0.3	[40●●]

Table 2 (continued)								
Application	Fruit	Effects	Irradiation dose (kGy)	Reference				
Compliance with phytosanitary regulations	Lychee	Control of the oriental fruit fly, <i>Bactrocera dorsalis</i> (sterility)	0.25	[41]				
	Mango	Control of fruit fly, Ceratitis capitata (sterility)	0.15	[42]				

irradiation can inactivate bacteria like *Escherichia coli* and *Salmonella* spp., reducing the risk of foodborne diseases [34]. Another study reported the use of GI as an intervention treatment to inactivate *Escherichia coli O157:H7* in freshly extracted apple juice [35••]. In blueberries, irradiation was used to reduce the levels of *Salmonella* spp., ensuring the safety of the fruit for consumption [33].

Improved Quality in Minimally Processed Fruit Products

In the case of minimally processed fruit products, irradiation can also serve the purpose of preservation [43]. The primary merit of employing this non-thermal technology relies on its proven effectiveness to maintain the quality and safety of the produce, preserving several compounds associated to quality attributes such as pigments, nutrients, bioactive compounds, and typical flavor [37, 44•]. When applied at optimized doses, GI proved effective to enhance the textural attributes of minimally processed fruit products [36], increase of their antioxidant capacity [38], and prevent physiological disorders by inhibiting the activity of polyphenol oxidase (PPO) and peroxidase (POD). These enzymes' activities are closely associated to deteriorative processes that lead to browning [7, 45].

GI as a Stress Elicitor Treatment

Low doses of gamma irradiation (0.5 kGy) were employed on papaya fruit as a way to induce ripening, rapidly attaining the fruit's commercial firmness [39]. Nevertheless, when samples are exposed to higher radiation doses (> 1.0 kGy), the elevated levels of ROS generated caused damage to the functionality of cellular materials. From a commercial perspective, the application of low doses (0.1 and 0.3 kGy) on minimally processed peaches significantly increased the softening rate, an effect that could be appreciated by a consumers panel [40••]. This is particularly relevant since stone fruits are typically harvested while still unripe and firm, for a proper postharvest handling. Therefore, GI can help reach faster the optimal firmness values for consumption, rendering a homogeneous product at the precise time that it is required.

Induction of the Physiological Mechanism of Defense in Fruit Products

Considering that GI can be classified as an abiotic stress, it would be reasonable to anticipate a physiological response to this condition [46]. The cellular response to stress involves, among others, the induced synthesis of heat shock proteins (HSP), which serve as a protective mechanism against thermal and oxidative stresses. This protection helps prevent cell death and enhances stress tolerance [47]. This phenomenon, well-documented in the context of tumor therapy, can apparently play a role in protecting tumor cells from stressinduced lethal damage [48], and has also been observed in bacteria [49, 50], although it has been relatively unexplored in plant tissues.

Typically, HSP are synthesized in cells of tissues exposed at low levels of stress, and the increase in their relative concentration is a fundamental aspect of the natural response of any living tissue to thermal stress [51]. In fact, the overexpression of these proteins has been recognized as an early marker of the response to the exposure of different types of stress [52] and has been associated with the enhanced resilience of heat-treated products against chilling injury [53].

The overexpression of HSP was detected in minimally processed peaches exposed to low irradiation doses of 0.1 and 0.3 kGy, a relevant finding not previously documented in fruits [40••]. Therefore, HSP could be used in plant tissues as a biomarker of the stress brought about by exposure to irradiation, capable of preventing physiological damage. This physiological tolerance appears to be a part of the evolutionary adaptation of plant tissues to different external disturbances [54, 55]. A better understanding of these phenomena can contribute to optimize GI applications and enhance the production of high-quality fruit products with extended shelf life.

Compliance with Phytosanitary Regulations

GI has been used to comply with phytosanitary regulations in the international trade of fruits. In mangoes, irradiation can be employed to control pests like fruit flies and meet export requirements of certain countries [42]. In lychees, irradiation has been applied to control the oriental fruit fly, *Bactrocera dorsalis*, ensuring fulfillment of export requirements [41].

Food irradiation has been approved by health and safety authorities in more than 57 countries, based on Codex Alimentarius standards. GI has a promising potential in developing countries, where food is often altered during the postharvest stage due to infrastructure deficiencies. In particular, Argentina has been a pioneer both in research studies and in keeping its legislation up to date. This technology is becoming increasingly relevant, given the modification of the Argentine Food Code [56] that promotes its application in different types of products, including fresh fruits. In this case, the maximum allowed doses are regulated according to the purpose of the irradiation: 1.0 kGy for delaying ripening, disinfecting insects, and quarantine control, and 2.5 kGy for controlling spoilage microorganisms. In June 2023, the Argentine regulatory agency (Servicio Nacional de Sanidad y Calidad Agroalimentaria - SENASA) approved, under Resolution 495/2023, minimum conditions for the application of ionizing energy treatments in regulated items for phytosanitary purposes: plant, plant product, storage place, packaging, means of transport, container, soil, and any other organism, object, or material capable of harboring or dispersing pests, which are considered commodities subjected to phytosanitary measures, especially in international transport [57].

It should be highlighted that the application of GI in food has been for long considered as a safe and effective treatment, not representing a health hazard, although the safety of their application for (reasonable) technological purposes must be demonstrated prior to their commercial use. The technology cannot be used as a substitute for good manufacturing practices (GMP) or good agricultural practices (GAP), or to reduce unacceptably high levels of microbial contamination [58].

Limitations and Prospective on the Use of GI

It is well known that the successful commercial implementation of any technology primarily depends on the availability of an adequate infrastructure. In this sense, one of the limitations of irradiation is the requirement of high capital costs, a minimum critical capacity, and a certain volume of product for the operation to be economically feasible. In contrast, it has the advantage of its low operating cost and minimal energy requirements [59]. In the case of fruit processing, it represents a treatment whose efficacy will depend on several factors, resulting in a high variability, which makes difficult its standardization. Thus, the successful implementation requires the establishment of cultivar aptitude; optimization of variables such as irradiation dose, degree of maturity, physiological state of the fruit, temperature, and atmosphere during and after treatment; and requirement of pre- and postharvest treatments, as well as the susceptibility and the potential microbial load in the product.

On the other hand, consumer attitudes towards irradiation are undergoing a positive change, mainly due to the development of pathogen resistance to various fungicides, the banner of some additives from the market, and the growing consumer demand for residue-free fresh produce. Therefore, this technology is considered as a promising alternative to improve the quality of fresh and fresh-cut produce. However, its acceptance still faces opposition from some consumers. This phenomenon could be due to economic and logistical factors, but also to psychological concerns of irradiation, due to the lack of public knowledge about the safety and wholesomeness of irradiated foods [4, 60].

Consequently, it would be necessary a more effective diffusion on the safety and the proven benefits of the application of irradiation in food. This could increase public understanding and lead to better consumer acceptance of irradiated products [61, 62]. In this sense, different initiatives have been proposed, such as the development of educational programs, which are expected to contribute to a better knowledge and attitude of consumers, and to positively influence the purchase decision of irradiated products [63].

Among the main factors that would contribute to a widespread application and economic feasibility of this technology, it can be mentioned the need of research funding from the government and industry, the development of new applications, a harmonized legislation, a better consumer attitude, and the practical solution of different economic and logistical issues associated to its use. Similarly to the use of genetic engineering technologies for food production, it is essential that the education of consumers and the compromise of professionals to solve the technological challenges be an integral part of future developments, together with the feasibility of industrial application of this technology.

A technology that was relatively obscure just two decades ago, is now firmly established itself as a key phytosanitary control, together with chemical and non-chemical methods, ensuring the biosafety of fresh fruit and vegetable products in international trade. The global trade of irradiated produce has increased to substantial levels. Irradiated fresh produce not only shows an appealing appearance and taste but also stands out for its absence of post-harvest treatment residues and affordability. This recognition has been widespread among growers, traders, retailers, and consumers in numerous countries. However, the question remains: will phytosanitary irradiation continue to expand at its current rate? The primary hurdle for its further growth seems to be non-science-based regulations. On the flip side, the simplicity of the irradiation process, its effectiveness, and its low environmental impact are compelling advantages for the future. To fully harness these benefits, it is imperative to maintain and broaden international cooperation in this field.

In addition, it will be essential to invest in production capacities, as well as to satisfy the demanding requirements of the international trade for compliance with food safety regulations, quality standards and innovative technologies related to the Industry 4.0 model.

Conclusions

The application of gamma irradiation to preserve food products represents an emerging and promising research area. Several studies reported the effects of this technology combined with other technologies such as modified atmosphere to remove microbial growth, maintain the quality parameters, and extend the shelf life of fruit and minimally processed fruit products. Further research is necessary to better understand the underlying physiological and molecular mechanisms induced by gamma irradiation against stress, which will help establish optimum processing conditions able to preserve pigments, vitamins, and bioactive compounds, as well as maximize the induction of certain proteins associated to the defense mechanism in plant tissues. In this direction, official regulations should be harmonized, and the process standardized, to increase the international trade of fruits reaching more distant markets, while protecting the health of consumers and reducing losses and waste.

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Declarations

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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