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Non-thermal plasma treatment as a new biotechnology in relation to seeds, dry fruits, and grains

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Abstract

Non-thermal plasma (NTP) technology offers wide potential use in the food technology, the same as in the unconventional agriculture. Some seeds, dry fruits, grains and their sprouts gain popularity in the culinary industry as 'raw seeds'. This review paper draws the current research and trends in NTP pre-treatment of selected seeds/fruits that are useable as 'raw seeds'. The main applications are connected with activation of seed germination, early growth of seedlings, microbial inactivation of seed/fruit surface, and possibility of increasing quantity of biological active compounds in sprouting seeds. The paper presents a list of plant species that are able to be used as 'raw seed' including current information about main type of NTP treatment implemented.

Keywords: biotechnology, early growth, plasma technology, low-pressure discharge, microbiological inactivation, seed modification

(Some figures may appear in colour only in the online journal)

1. Introduction

Seeds/fruits are rich in nutrients and have many health benefits due to the fact that they are abundant sources of bioactive compounds including phytochemicals (phenols, flavonoids, and carotenoids), vitamins (vitamin C, folate, and pro-vitamin A), minerals (potassium, calcium, and magnesium), proteins, and fibres [1, 2]. These components are linked to considerable evidence for nutritional benefits as well as the reduction of the risk of cardiovascular diseases and cancer [3–5]. Numerous studies have shown that different types of seeds/fruits (and nuts) can actually prevent weight body gain, the development of heart disease and the accumulation of LDL cholesterol [6].

Some seeds/fruits are being used increasingly as a feed ingredient for both humans and domestic animals. Many seeds, grains, and dry fruits can be incorporated easily into various diets and recipes. In many diets, only organic seeds/fruits, in their raw state (natural), are recommended. Sprouting of seeds significantly improves their digestibility [7, 8]. During the seed germination, the seed receives water and metabolism starts to activate more enzymes and organic matters. Starch breaks down into simple sugars, proteins into amino acids, and fats into free fatty acids [9]. Content of vitamins (especially B, C, E, A) may be increased up to several times [10, 11] and naturally-occurring anti-nutritional factors may be decreased during seed sprouting [8]. For example, seed germination of red cabbage and broccoli brought significant increases of total phenol compounds and concentration of melatonin [12]. These information are valuable for the incorporation of 'seeds' and sprouted seeds into the diet to promote potential health benefits.

Unfortunately, fresh plant products are recognized as important holders for transmission of various pathogens. Microbial contamination can be infected during many steps:

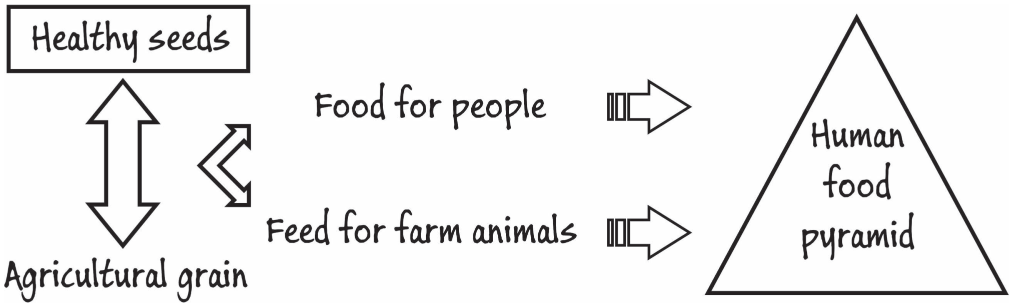

Figure 1. Seeds as grains in agriculture, people foodstuff, and feed for animals enter into human food pyramid.

growing in fields, processing on farms, post harvest processing, transportation, storage, and distribution [13, 14]. Raw plant products usually contain only natural microorganisms, which are not human pathogenic. Nevertheless, human illness associated with consuming fresh agricultural plant products (above all tomatoes, lettuce) have been reported [14, 15]. Some microorganisms may reside in protected sites on surface of the fresh plant products and are able to survive long time in the resting phase [14, 15]. For this reason, the precautionary principle is important in the case of food contamination entering the human food chain. Figure 1 presents how closely the agriculture production of seeds/fruits is related to the human food pyramid; two direct links and one indirect are obvious. The need of 'healthy seeds' for human nutrition is essential. Seeds/fruits traditional consumption as raw food and the use in modern diets is appropriate.

Green biotechnology applied to agricultural processes, produces more environmentally solutions alternative to traditional agriculture. The biotechnology enables improvements that are not possible with traditional way. Demands for current foods are increasing, they include increasing yield, disease and pest resistance, drought resistance, good aroma, and taste. One of the goals is tackle for secure food including the reduction of environmental footprint of chemicals.

Plasma technology, as a modern technique, is one of many currently studied options for improving both food [16, 17] and seeds/fruits quality [18]. In this article, our goal is to present overview about plasma technology and its potential applications for human pathogen decontamination on fresh seeds/fruits. It has been found that non-thermal plasma (NTP) improves germination parameters and inactivates the contaminated surface of seeds/fruits from various microorganisms. This paper summarizes current research and brings a wider introduction to the subject of raw seed.

2. Plasma technology

Physical plasma is considered to be the fourth state of matter and it constitutes the major part of the visible Universe. Due to the use of constant energy by stripping electrons, plasma is naturally energetic. They can exist in a wide range of temperatures without changing state, unlike ordinary matter. It is not a human invention, and it is present in the nature, as 'fire' in the Sun, stars, sparks and as flashes of lightning [19]. A number of methods of plasma generation have been possible classifying with many criterions: electrical current waveform (direct current, radio frequency, pulse etc), electrodes geometry (e.g. surface discharge, spot discharge plasma), and experiment geometry (e.g. after glow plasma) [20, 21].

Plasma as same as gas particles do not have definitive shape. However, the presence of electric and magnetic field shape it into a different structure. On the basis of relative temperatures of the ions, neutrals, and electrons, plasma is categorized as 'thermal' or 'non-thermal.' Electrons and heavy particles of thermal plasma remain in thermal balance with each other. NTP is not in thermodynamic equilibrium, either because the ion temperature is different from the electron one [22]. The ions and neutral particles of NTP remain at room temperature, whereas electrons are hotter.

Plasma needle and pencil, dielectric barrier discharge, corona discharge, arc plasma device, and many others apparatuses are being used for the production of NTP. The diversity of the laboratory devices, many variable plasma parameters, and a lack of standard treatment procedures made the comparison of them almost impossible [16, 23].

3. Microbe sterilization with NTP

The parametric study of plasma for sterilization is of importance in understanding and controlling the deactivation of microbes, because the main sterilizing factors are strongly dependent on the plasma source type and/or the plasma characteristics [24]. Recent literature has shown the applications of NTP for sterilization of various surfaces in medical and dental equipment [16, 24], packaging in food industry [16, 25]. Nowadays, atmospheric pressure NTP is more frequently used for the sterilization of both living and non-living materials [16, 23, 24, 26, 27]. It is distinguished by a low degree of ionization, tissue tolerable temperature, and atmospheric pressure. It was found that many microbes are inactivated after NTP treatment: bacteria [15, 28, 29], fungi [30, 31], and viruses [32]. The idea to use plasma to sterilize the surface of seeds/fruits is about 10 years old (e.g. [30, 33–37]).

I one project, NTP (air gas and sulphur hexafluoride) was used for decontamination of hazelnuts (*Corylus* sp.), peanuts (*Arachis hypogaea*) and pistacio (*Pistacia vera*) against *Aspergillus parasiticus* and aflatoxins at the nuts surfaces

Table 1. List of plant species which seeds/fruits served as a tested biological material in experiments with non-thermal plasma discharges. Seeds/fruits presented are able to be used as 'raw seeds'.

Common name of plant species	Latin name	Taxonomy (family)	[a]Part useable as 'raw seed'	Plasma source/device	Aim of study	References
Alfalfa	*Medicago sativa* L.	Fabaceae	Seed	NTP	Germination	[44]
Barley	*Hordeum vulgare* L.	Poaceae	Caryopsis	Continuous and pulsed glow discharge plasmas	Germination + early growth	[45]
				Low pressure cold plasma	Decontamination of *Aspergillus* app. and *Penicillium* + germination	[30]
				Glow discharge plasma	Germination + early growth + fungal deactivation	[46]
				GlidArc plasma + downstream microwave plasma	Germination + early growth	[47]
Bean	*Phaseolus vulgaris* L.	Fabaceae	Seed	Cold radiofrequency plasma	Wetting properties + imbibition + germination	[48]
				Cold radiofrequency air plasma	Wetting properties + germination	[49]
				Low pressure cold plasma	Decontamination of *Aspergillus* app. and *Penicillium* + germination	[30]
				Rotating plasma reactor (+coating of seeds)	Germination	[40]
Broccoli	*Brassica oleracea* var. *italica*	Brassicaceae	Seed	Corona discharge plasma jet	Decontamination + sprouting	[50]
Brown mustard	*Brassica juncea* (L.) Czern.	Brassicaceae	Seed	NTP	Germination	[51]
Buckwheat	*Fagopyrum esculentum* Moench	Polygonaceae	Seed	GlidArc plasma + atmospharic pressure NTP with a planar rotating electrode + downstream microwave plasma + surface dielectric barrier discharge	Germination + early growth	[52]
Fenugreek	*Trigonella foenum-graecum* L.	Fabaceae	Seed	NTP	Germination + fungal reduction	[53]
Hemp	*Cannabis sativa* L.	Cannabaceae	Achenium	GlidArc plasma + downstream microwave plasma	Germination + early growth	[54, 55]
Chickpea	*Cicer arietinum* L.	Fabaceae	Seed	NTP	Microbial reduction + germination	[33]
				Low pressure NTP	Fungal decontamination	[30]
				NTP	Germination + fungal inactivation	[53]
Lamb's quarters	*Chenopodium album* L.	Amaranthaceae	Achenium	NTP	Dormancy breaking	[56]
				NTP	Germination + early growth	[57]
				NTP	Germination	[58]
Lentil	*Lens culinaris* Medik.	Fabaceae	Seed	Cold radiofrequency air plasma	Wetting properties + germination	[49]
				Low pressure cold plasma	Decontamination of *Aspergillus* app. and *Penicillium* + germination	[30]
Maize, corn	*Zea mays* L.	Poaceae	Caryopsis	Diffuse coplanar surface barrier discharge	Early growth + physiology	[59]
				Low pressure NTP	Fungal decontamination	[30]

Table 1. (Continued.)

Common name of plant species	Latin name	Taxonomy (family)	[a]Part useable as 'raw seed'	Plasma source/device	Aim of study	References
				Glow discharge plasma	Germination + early growth + fungal deactivation	[46]
				Rotating plasma reactor (+coating of seeds)	Germination	[40]
				diffuse coplanar surface barrier discharge	Imbibition + physiology	[60]
				NTP	Germination + early growth	[61]
				GlidArc plasma + downstream microwave plasma	Germination + early growth	[47]
Mung bean	*Vigna radiata* (L.) R. Wilczek	Fabaceae	Seed	Atmospheric-pressure N_2, He, air, and O_2 microplasma arrays	Germination + early growth + catalase activity	[62]
				Cold plasma	Germination + early growth + surface change + enzymatic activity	[63]
Oat	*Avena sativa* L.	Poaceae	Caryopsis	Downstream microwave plasma	Germination + early growth	[64]
				Continuous and pulsed glow discharge plasmas	Germination and early growth	[45]
				Low pressure NTP	Fungal decontamination	[30]
				GlidArc plasma + downstream microwave plasma	germination + early growth	[47]
Pea	*Pisum sativum* L.	Fabaceae	Seed	Dielectric barrier discharge	Physiology + germination	[65]
				Diffuse coplanar surface barrier discharge	Surface + germination + early growth + physiology	[66]
				Rotating plasma reactor (+coating of seeds)	Germination	[40]
Poppy	*Papaver somniferum* L.	Papaveraceae	Seed	Downstream microwave plasma	Germination + early growth	[67]
				GlidArc plasma + downstream microwave plasma	Germination + yield	[68]
Quinoa	*Chenopodium quinoa* Willd.	Amaranthaceae	Achenium	Atmospheric and low pressure NTP	Surface change + germination	[69]
Radish	*Raphanus sativus* L.	Brassicaceae	Seed	Cold atmospheric plasma	Germination + early growth	[70]
				NTP	Early growth	[71]
				Rotating plasma reactor (+coating of seeds)	Germination	[40]
				Corona discharge plasma jet	Bacteria decontamination + sprouting	[72]
				Dielectric barrier discharge (+activation of water)	Germination + early growth	[73]
				Large-volume atmospheric glow discharge (+activation of water)	Yield	[74]
				Dielectric barrier discharge	Early growth	[75, 76]
				Low pressure O-2 radio frequency discharge plasma irradiation	Early growth	[77]
				Atmospheric pressure NTP (He) + low pressure O-2 radio frequency discharge plasma	Early growth	[78]

Table 1. (Continued.)

Common name of plant species	Latin name	Taxonomy (family)	[a]Part useable as 'raw seed'	Plasma source/device	Aim of study	References
Oilseed rape	*Brassica napus* L.	Brassicaceae	Seed	NTP	Germination under drought stress	[79]
				Corona discharge plasma jet	Bacteria decontamination + sprouting	[80]
				GlidArc plasma	Germination + early growth + physiology	[81]
				Dielectric barrier discharge + microwave plasma processed air	Bacteria inactivation + seed viability	[82]
Rice	*Oryza* sp.	Poaceae	Caryopsis	NTP	Imbibition + bacteria reduction + physiology	[83]
				NTP	Sterilization + germination	[84]
				Low-pressure NTP	Imbibition + germination + early growth + physiology	[85]
				Dielectric barrier discharge	Germination + early growth + antifungal activity	[86]
Soybean	*Glycine max* (L.) Merr.	Fabaceae	Seed	NTP (Ar)	Germination + early growth + physiology	[87]
				NTP	Germination + early growth + physiology	[88]
				Low pressure NTP	Fungal decontamination	[30]
				Rotating plasma reactor (+coating of seeds)	Germination	[40]
Wheat	*Triticum* sp.	Poaceae	Caryopsis	Diffuse coplanar surface barrier discharge	Germination + early growth + fungal inactivation	[34]
				NTP	Fungal reduction	[89]
				Surface discharge reactor	Germination + early growth	[90]
				Cold helium plasma	Germination + early growth + yield	[91]
				Cold radiofrequency air plasma	Wetting properties + germination	[49]
				Downstream microwave plasma	Surface + germination + early growth + physiology	[64]
				Dielectric barrier discharge	Surface + germination + early growth + physiology	[92]
				Low pressure NTP	Fungal decontamination	[30]
				GlidArc plasma + downstream microwave plasma	Germination + early growth	[47]
				Dielectric barrier discharge	Bacterial inactivation + physiology	[93]

[a] Types of fruits are presented in latin terminology.

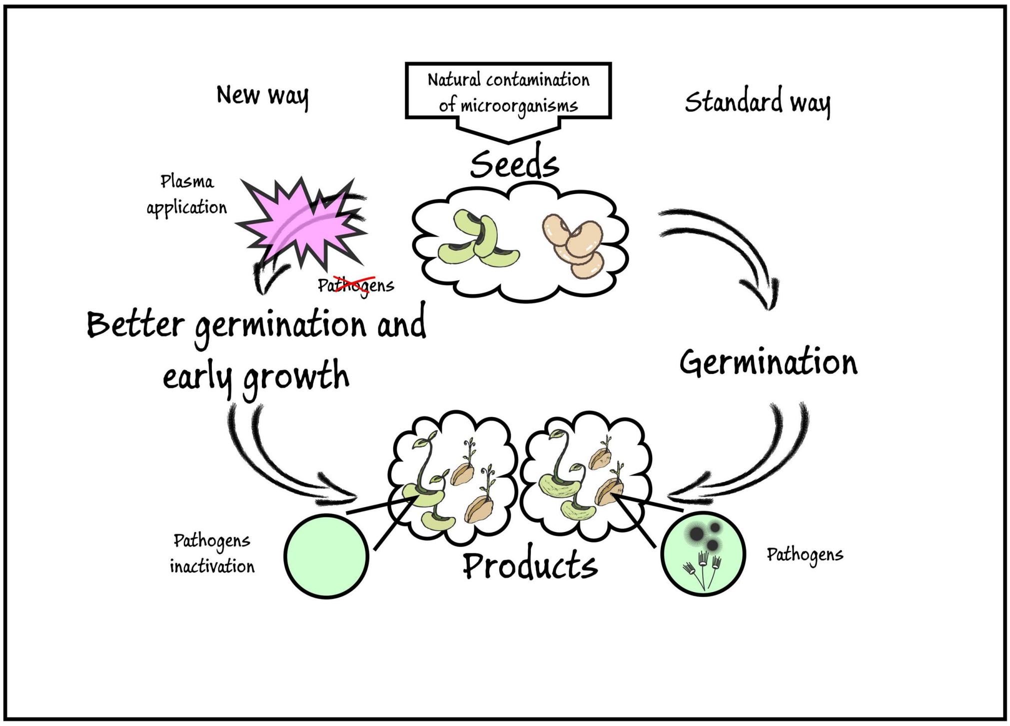

Figure 2. The scheme presents the principle of treated seeds with non-thermal plasma without microbial pathogens and with better germination parameters.

[35]. In other experiment, seeds/fruits of wheat (*Triticum durum*), bean (*Phaseolus vulgaris*), chick pea (*Cicer arietinum*), soybean (*Glycine max*), barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*), lentil (*Lens culinaris*), and maize (*Zea mays*) were contaminated with spores of *Aspergillus parasiticus* 798 and *Penicillium* MS1982, and then treated by NTP [30]. Depending on the seeds/fruits surface, plasma gas type, plasma treatment time and power, and the microbial population density, the percentage of seeds/fruits infection was reduced to below 1%, without reducing the seeds/fruits quality below the commercial threshold of 85% seeds/fruits germination under described plasma conditions [30].

A significant reduction of the seed borne microbial contamination was observed after 120 and 300 s of NTP treatment in seeds of chick pea (*Cicer arietinum*) [33]. In another experiment [34], effect of NTP on wheat seeds artificially infected with pure cultures of filamentous fungi isolated from surfaces of untreated wheat seeds was determined on dead wheat. The result shows the efficiency of the treatment of seeds in the following decreasing order: *Fusarium nivale* > *F. culmorum* > *Trichothecium roseum* > *Aspergillus flavus* > *A. clavatus*.

Based on the above, NTP may inactivate wide spectrum of microbes on the seeds/fruits surface. However, the mechanism through which NTPs inactivate living cells is not well known. Several plausible hypotheses exist, including chemical etching of the cellular surface, disruption of cellular electrostatic potential, or oxidative intracellular damage [38]. The lack of a standard protocol for preparing and treating cells with plasma, in addition to technical limitations associated with analysing cells on surfaces rather than in solution, represent critical impediments for determining the exact mechanism through which NTP inactivates microbes [39].

4. Seeds/fruits activation with NTP

The idea of activating seeds/fruits germination precedes their attempts of sterilization (e.g. [16, 40–43]). It is a usable effect, that atmospheric pressure NTP treatment may start better germination and early growth in many seeds/fruits (table 1).

Increasing of the germination characteristics is connected with better water intake. It was found, that plasma treatment eroded seeds/fruits surface, which is then able to receive water faster [36, 49, 64, 88]. Above all, it seems, seeds/fruits positive reaction is probably caused by a 'short stress' during plasma treatment. Penetration of active species from plasma through seed coat inside seed effects metabolism of the seed germination and seedling growth [64, 94]. It acts on the physiological processes of germination. At high plasma exposure there are stress reactions and reduced germination (and death of the seed). If the plasma treatment is adequate for the species, it is beneficial. Better germination usually consists in a bigger number of germinating seeds and in a shorter germination time. Seedlings growing from plasma pre-treated seeds/fruits are usually confirmed by a larger root system or larger weight of the whole ones.

The seeds/fruits of some plant species usually germinate well after NTP treatment, e.g. some grains [45], lamb's quarters (*Chenopodium album* agg.) [56, 57], poppy (*Papaver* *somniferum*) [67], maize (*Z. mays*) [59], pea (*Pisum sativum*) [66], oilseed rape (*Brassica napus*) [80], and black mulberry (*Morus nigra*) [95], but some do not, e.g. oat (*Avena sativa*) [64] and Smirnow's rhododendron (*Rhododendron smirnowii*) [95]. So, plant species (cultivars including) and type of the seeds/fruits are important factors. Significant differences in response to NTP treatment were found among different cultivars of hemp seeds (*Cannabis sativa*) [55].

The type of plasma apparatus/discharge and the parameters of the plasma treatment are crucial too. Significant differences were found in the germination and early growth of buckwheat (*Fagopyrum esculentum*) [52] and hemp [55] when different apparatus were used.

Some experiments have been focused on content of biologically active compound. Mildaziane and collectives [94] found, that seedlings of purple coneflower (*Echinacea purpurea*), grown from achene with NTP treatment, had contents of vitamin C and phenolic acids substantially higher in comparison to the control. Seeds/fruits treatments induced large increase in radical scavenging activity (up to 114%) in leaf extracts.

The optimal solution for the raw seeds/fruits technology would be surface disinfection, good germination and growth, but also maintaining or increasing the amount of bioactive and nutritional substances (figure 2). So, optimization of NTP conditions in plasma technology before seeds/fruits sprouting is crucial for the greatest biological effectiveness.

5. Conclusion

Published research results show interesting effects of NTP on seeds/fruits from the point of view of food and medicinal usefulness. NTP appears to be a powerful and useful technology for the both seeds/fruits growing stimulation and seeds/fruits surface decontamination and disinfection. Current research looks for standard plasma parameters to inactivate microorganisms while preserving the viability of seeds/fruits. A number of experimental studies related to seeds/fruits treatment have been performed, where various background plasma generators (devices, apparatuses) have been tried. Some raw seeds/fruits can become a more acceptable part of a regular, healthy diet in the future. NTP applications in agriculture and food industry are undergoing fast development and plasma technology is becoming an important part of modern healthy foodstuff. The tools of plasma technology offer both a challenge and tremendous opportunity.

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