Novel Technologies for Enhancing Food Safety, Quality, and/or Nutrition of Fruits and Vegetables: Pulsed Light and Cold Plasma as Examples

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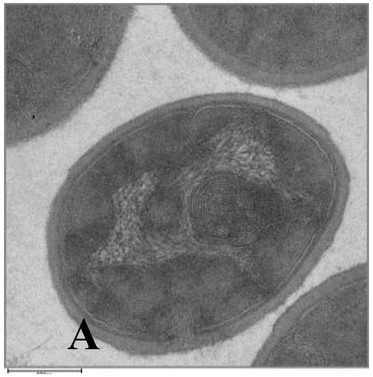
Illinois Institute of Technology

Traditional Technologies



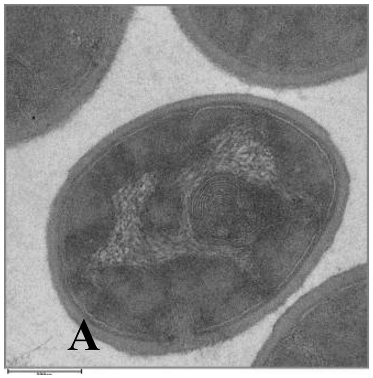
Destruction of
Enzymes
Pathogenic microorganisms
Spoilage microorganisms

VS

Image source: Gila Brand at en.wikipedia

Retention of
Color
Flavor
Nutritients
Texture

Novel and Emerging Technologies



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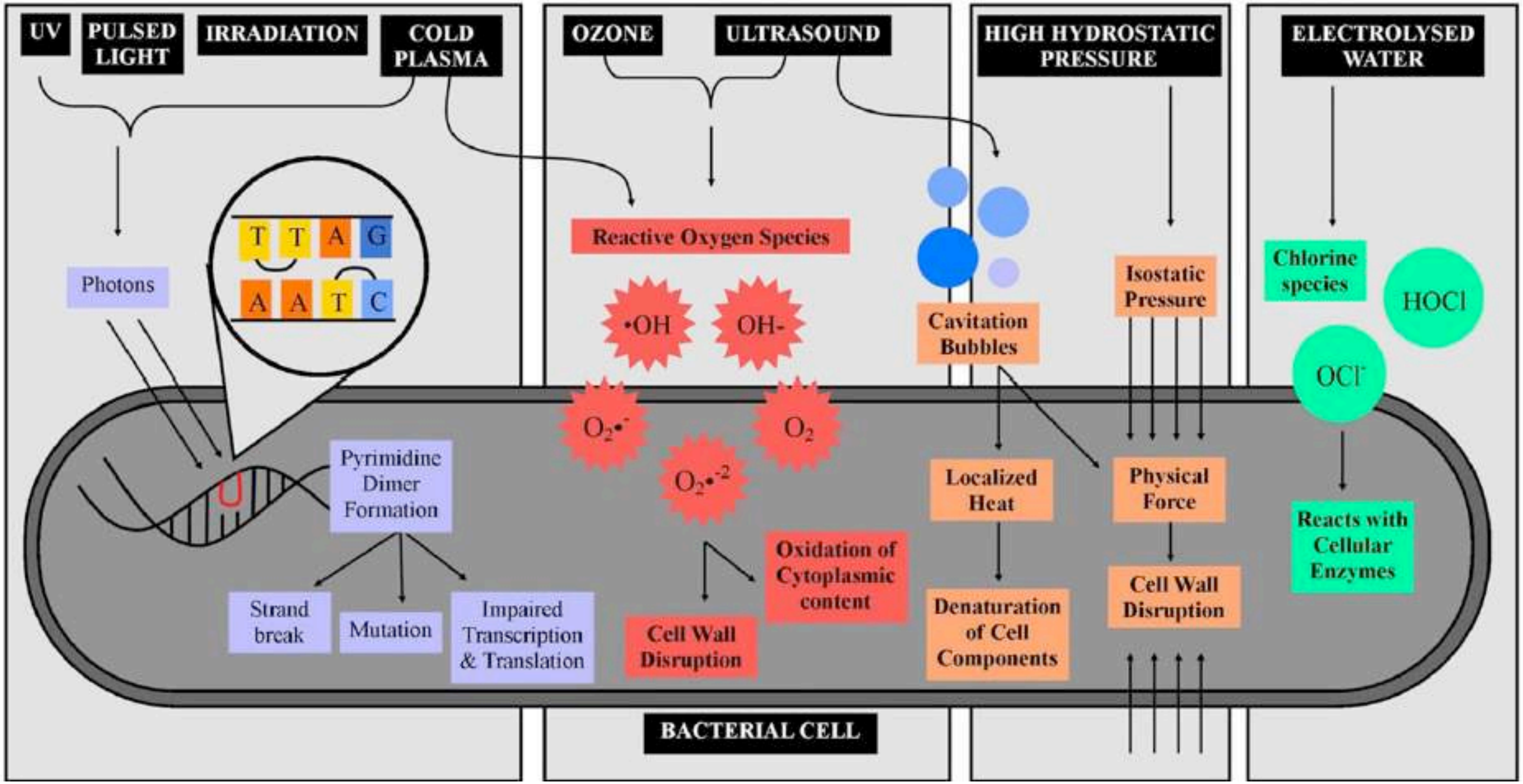


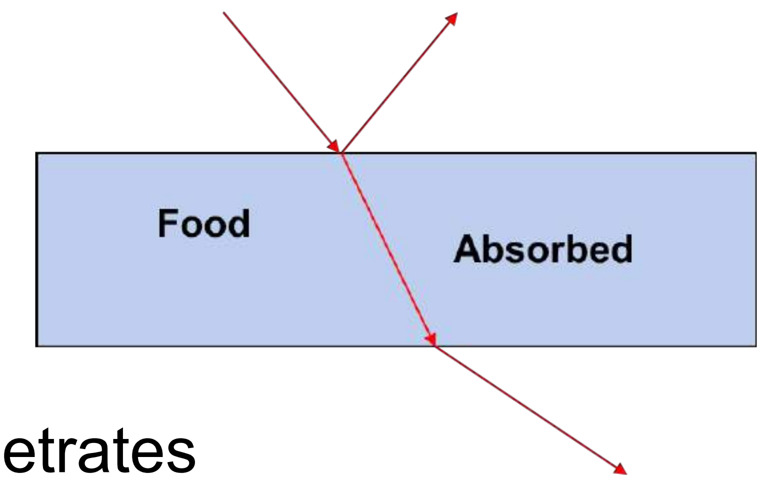
Source: Bhilwadikar et al., 2019, Comprehensive reviews in food science and food safety. Available online at

Potential applications of light based technologies

- Surface decontamination of foods
- Surface decontamination of equipment and food preparation surfaces
- Pasteurization of water and transparent liquid foods
  - Under some conditions, pasteurization of opaque liquids
    - Turbulence
    - Thin film reactor
- Reduction of allergenicity in foods such as peanuts, treenuts, etc. (more studies needed)
- Vitamin D enrichment in selected foods
- Enrichment of different bioactive compounds
- Can be potentially used in combination with other technologies – hurdle approach
- Toxin reduction, reduction in herbicide (more studies need to be conducted to understand)

Fundamentals

$$E = h\upsilon = \frac{hc}{\lambda}$$

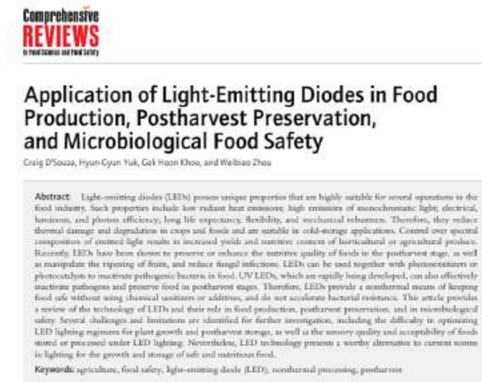


- Intensity decreases as light penetrates

$$I = TI_o e^{-X}$$

- T is the transparency coefficient of the food material
- I is the intensity of light at a distance x from the surface
- I_o is the initial intensity of UV-light, and
- x is the distance below the food surface

LED (Light-Emitting Diodes)


Comprehensive REVIEWS

Application of Light-Emitting Diodes in Food Production, Postharvest Preservation, and Microbiological Food Safety

Craig D'Souza, Hyun-Gyun Yuk, Gek Hoon Khoo, and Weibiao Zhou

Abstract Light-emitting diodes (LEDs) possess unique properties that are highly suitable for several operations in the food industry. Such properties include low radiant heat emissions; high emissions of monochromatic light; electrical, luminous, and photon efficiency; long life expectancy, flexibility, and mechanical robustness. Therefore, they reduce thermal damage and degradation in crops and foods and are suitable in cold-storage applications. Control over spectral composition of emitted light results in increased yields and nutritive content of horticultural or agricultural produce. Recently, LEDs have been shown to preserve or enhance the nutritive quality of foods in the postharvest stage, as well as manipulate the ripening of fruits, and reduce fungal infections. LEDs can be used together with photosensitizers or photocatalysts to inactivate pathogenic bacteria in food. UV LEDs, which are rapidly being developed, can also effectively inactivate pathogens and preserve food in postharvest stages. Therefore, LEDs provide a nonthermal means of keeping food safe without using chemical sanitizers or additives, and do not accelerate bacterial resistance. This article provides a review of the technology of LEDs and their role in food production, postharvest preservation, and in microbiological safety. Several challenges and limitations are identified for further investigation, including the difficulty in optimizing LED lighting regimens for plant growth and postharvest storage, as well as the sensory quality and acceptability of foods stored or processed under LED lighting. Nevertheless, LED technology presents a worthy alternative to current norms in lighting for the growth and storage of safe and nutritious food.

Keywords: agriculture, food safety, light-emitting diode (LED), nonthermal processing, postharvest

- Still at its infancy
- There is a lot of interest
- Light (405 nm or around that range) → excitation of photosensitizer molecules within the microorganisms → generation of reactive species like singlet oxygen
- Applications
  - Inactivation depends upon the wavelength and the energy
  - Can preserve or enhance nutritional quality
  - Control of ripening of fruits
  - Reduce fungal infections during the growth of the plants
- Major disadvantage
  - Treatment time can often be very long (minutes to hours)
    - Currently more powerful systems are being developed to overcome this challenge

Inactivation Mechanism: Pulsed Light

- Photochemical
  - Formation of pyrimidine dimers
- Photothermal
  - Heat generated by infrared wavelengths causes localized heating
- Photophysical
  - Physical disruption of the cell wall from repeated "shocks"
  - Takeshita et al. (2003) observed structural damage in yeast by pulsed light

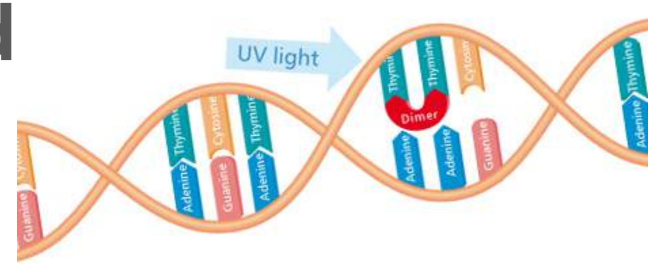


Image source: Steril innovations

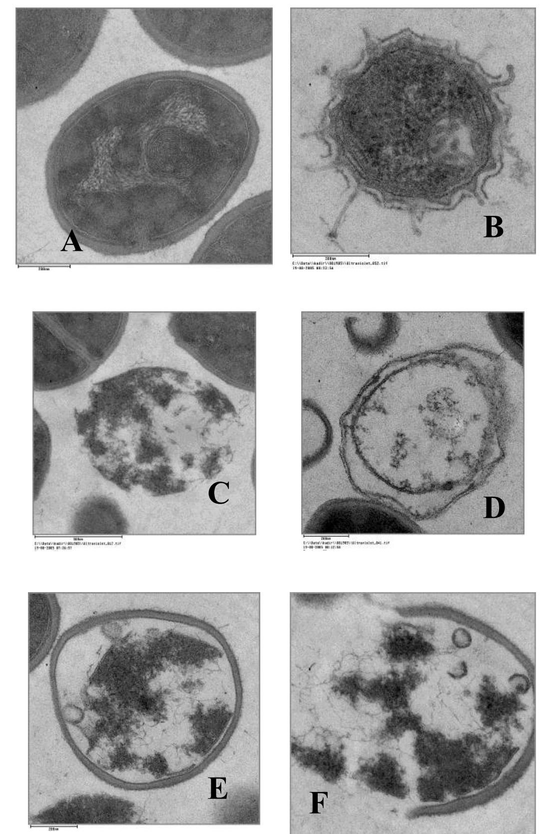


Image: Staphylococcus aureus cells in phosphate buffer treated with a 5 s pulsed light treatment (Krishnamurthy et al., 2009)

Inactivation Mechanism: UV light and LED light

- UV light
  - Photochemical
    - Formation of pyrimidine dimers
  - UV-A
    - affects bacterial cells by causing membrane damages and/or generate active oxygen species or H_2O_2
- LED (405 nm)
  - Photoexcitation of intracellular porphyrin molecules → reactive oxygen species → membrane and other damages (more research is being done to better understand)

Factors affecting inactivation

- Optical properties of the food product
  - Penetration depth
- Surface characteristics of the food
- Microorganisms
  - Strain, growth stage, growth method etc.
- Presence of suspended particulates
  - Shadowing effect
- Treatment time
- Distance
- Wavelength
- Temperature
- Intensity of the light
- Other factors relevant to each technology
  - Example: Pulsed light - Input voltage, frequency, energy per pulse, pulse duration

Effect of light based technologies on food quality

- In-package pulsed light treatment of bread slices → fresh appearance for two weeks (control – mold growth) (Rice 1994)
- Tomatoes treated with pulsed light – acceptable quality up to 30 days when refrigerated (Rice 1994)
- Sensory changes
  - Cabbage treated with pulsed light: plastic off-odor; faded after couple of hours
  - Pulsed light treated iceberg lettuce received better scores than the control samples for off-odor, taste, and leaf edge browning.
- In general
  - Quality changes depends on food and treatment time
- Most of the conditions DO NOT adversely affect the quality attributes

Pulsed light treated raspberry & strawberry

- Raspberry
  - E. coli O157:H7: 3.9 log CFU/g reduction (72 J/cm^2)
  - Salmonella: 3.4 log CFU/g reduction (59.2 J/cm^2)
- Strawberry
  - E. coli O157:H7: 2.1 log CFU/g reduction (25.7 J/cm^2)
  - Salmonella: 2.8 log CFU/g reduction (34.2 J/cm^2)

Untreated | 3 cm for 60 s

Source: Bialka, 2007

5 cm for 30 s | Untreated | 13 cm for 60 s

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Pulsed light treated blueberries

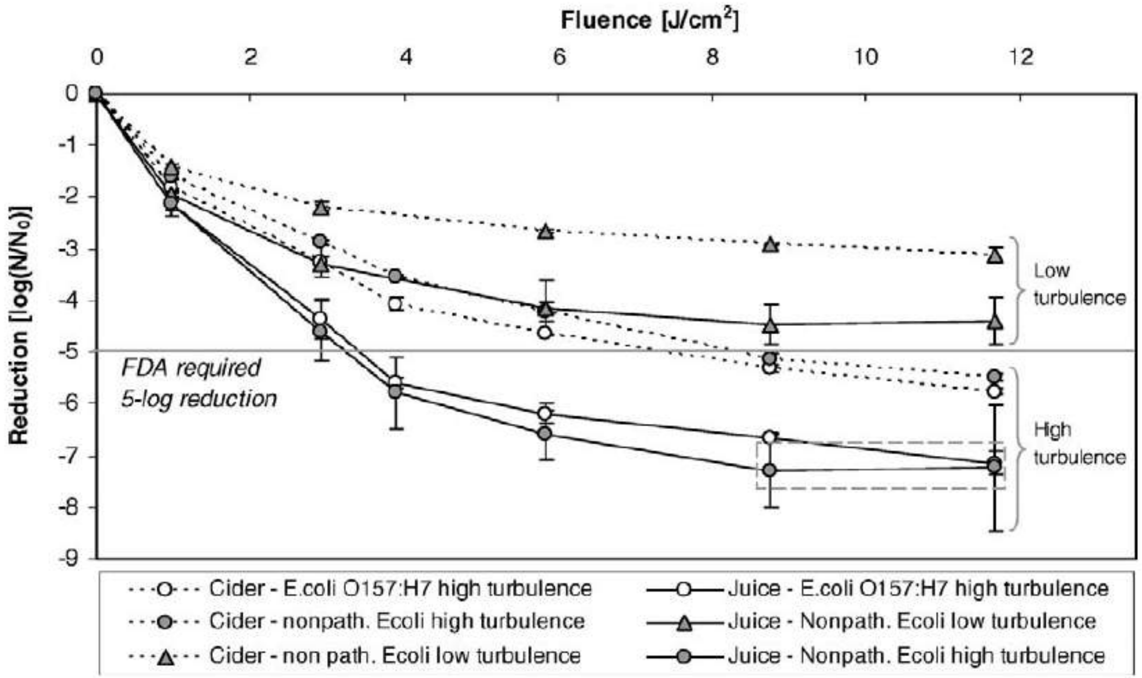
- Raspberry
  - E. coli O157:H7: 2.9 log CFU/g reduction (8 cm distance, 60 sec)
  - Salmonella: 4.3 log CFU/g reduction (8 cm distance, 60 sec)
- No change detected during sensory evaluation
- Source: Bialka et al., 2007

Treated at full voltage

Treated at 2,400 V

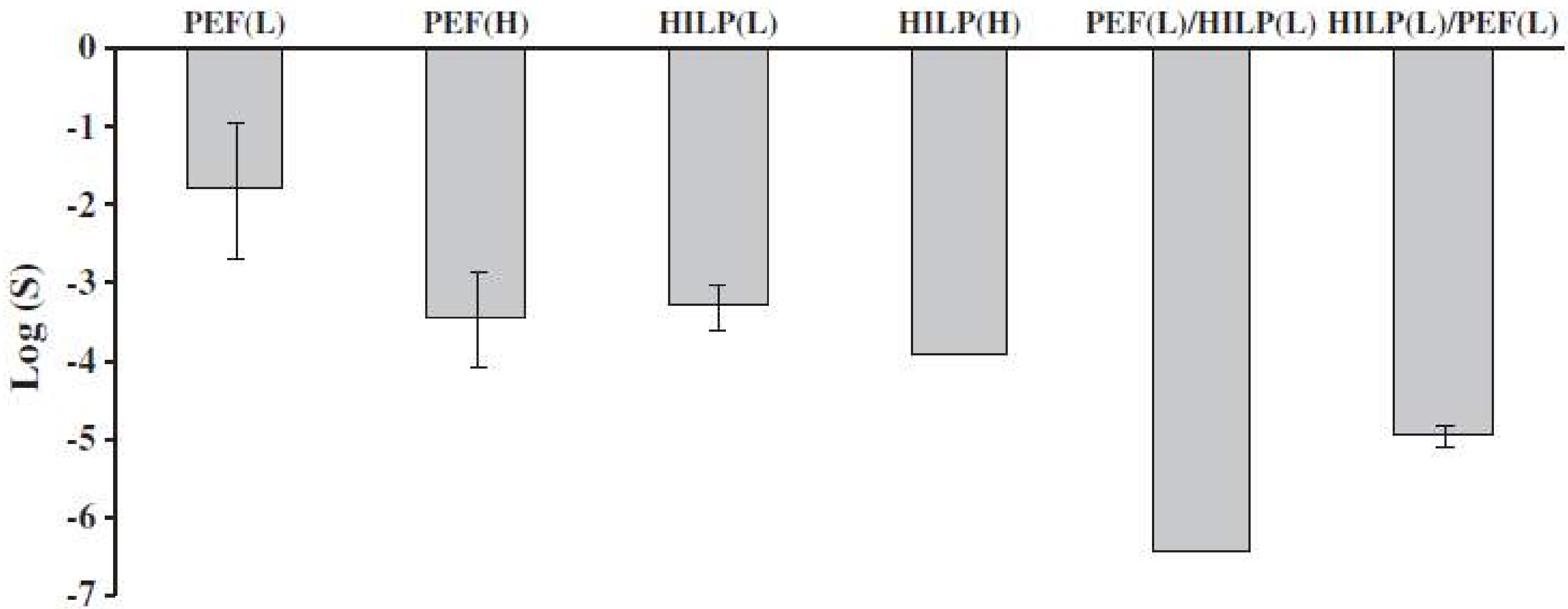
Pulsed light: Fruit juices

- Apple Juice (continuous treatment at 4 J/cm^2 – Pataro et al. 2011)
  - 4.00 $\log_{10}$ reduction of *Escherichia coli* and
  - 2.98 $\log_{10}$ reduction of *Listeria innocua*
- Orange Juice (continuous treatment at 4 J/cm^2 – Pataro et al. 2011)
  - 2.98 $\log_{10}$ reduction of *Escherichia coli* and
  - 0.93 $\log_{10}$ reduction of *Listeria innocua*
- Turbulent flow (Sauer and Moraru, 2009)



Hurdle approaches: An example

- Pulsed light + pulsed electric field treatment in apple juice
  - Resulted in > 5 log reduction of *E. coli* in most of the tested conditions when used in combination
  - Sequence matters



UV light

- Well developed and various references are available (Ex: Ultraviolet Light in Food Technology: Principles and Applications, CRC Press)
- Commercially used for treatment of
  - Apple cider (Cidersure®)
  - Milk (Surepure)
  - Other juice products
- Fresh cut fruits and vegetables
  - Water melon, apple, cantaloupe, pear, etc.
- Can be used for reduction of patulin mycotoxin

LED light (~405 nm)

- Salmonella on cut papaya → 0.3 - 1.3 log CFU/cm^2 reduction at chilling temperatures (LED treatment for 36 to 48 hours – 1.3 to 1.7 kJ/cm^2) (Kim et al., 2007a)
  - No change in physiochemical and quality attributes
- *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. On fresh cut mango – 1.0 to 1.6 log CFU/cm^2 reduction at 4 & 10°C (LED treatment for 36 to 48 hours – 2.6 to 3.5 kJ/cm^2) (Kim et al., 2007b)
  - LED treatment did not affect color, antioxidant capacity, ascorbic acid, β-carotene, and flavonoid

Light based technologies for nutrition

- Phenolic and other bioactive compounds
  - Generated by plants to protect the DNA when exposed to UV light
  - Increase in phenolic acids, anthocyanin, and flavonols in primarily on fruit peels (in flesh as well)
- Effects can be increased significantly with pulsed light
  - Example: vitamin D_2 content increases significantly with a 1 second pulsed light treatment
    - White button mushrooms - 724%
    - Brown button mushroom - 746%
    - Shiitake mushroom – 1200%
    - Oyster mushroom – 1618%

Regulatory approval

- Pulsed light
  - Approved by FDA in 1996
  - Can be used the decontamination of food or food contact surfaces, at cumulative doses below 12 J/cm^2 with pulse duration less than 2 msec
  - Food Code 21CFR179.41
- Ultraviolet
  - "Without ozone production: high fat-content food irradiated in vacuum or in an inert atmosphere; intensity of radiation, 1 W (of 2,537 A. radiation) per 5 to 10 ft"
  - Juice: "Turbulent flow through tubes with a minimum Reynolds number of 2,200"
  - Food Code 21CFR179.39

Challenges

- For some applications, the approved dose might not be sufficient
  - Petition FDA to increase the dose
- 3-dimensional exposure is key
  - shadowing effect if suspended solids are there
- Effect of various factors on the efficacy has to be well understood
  - Example: Pulsed light (other factors such as frequency, energy/pulse, etc.)

Key Takeaways

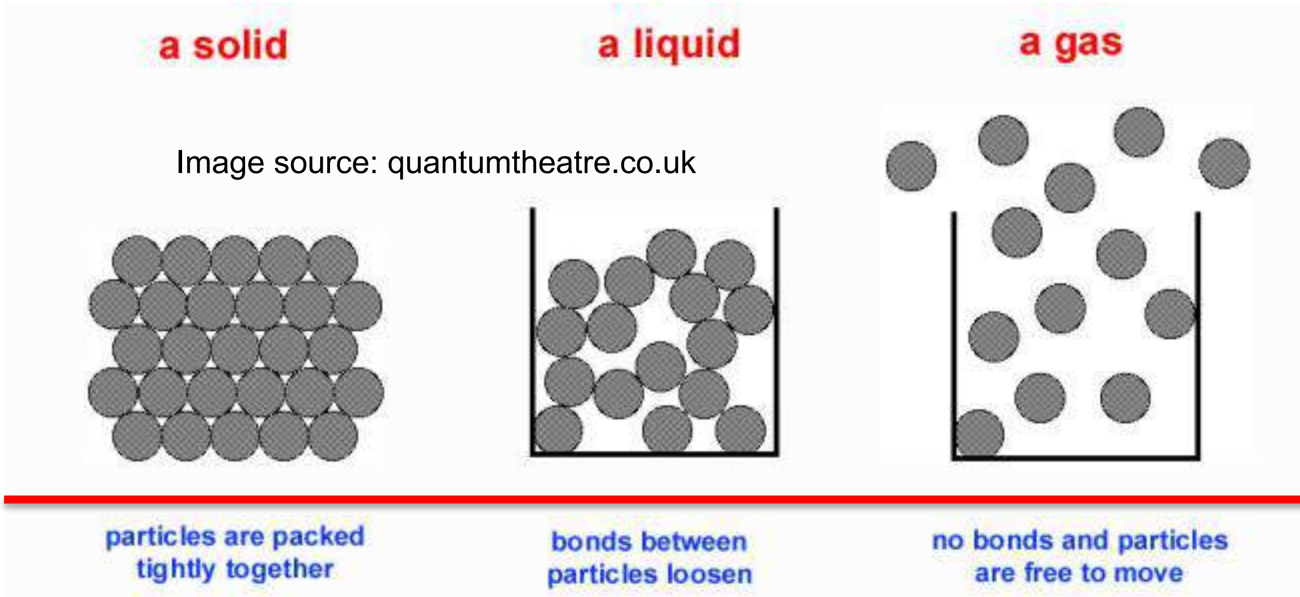
- Light based technologies are very powerful for selected applications
- Applications depend upon type of food, type of contamination (surface or internal), 3-dimensional exposure along with myriad factors
- Some of the technologies are still in its infancy → more research needs to be done
  - To better understand the technologies
  - To implement the technologies in real world applications
- Positive aspects
  - Already used in many large scale non-food applications → Easy to scale up and implement
  - Very easy to integrate with the existing setup like conveyor belts

What is cold plasma?

- Highly energetic form of partially ionized gas with a net neutral charge **at or near room temperature**
- Plasma consists of highly reactive species (~75) → 500 reactions
  - Gas molecules
  - Atoms
  - Charged ions
  - Free radicals (hydroxyl, superoxide and nitrogen oxides etc.)
  - Free electrons
  - Ozone
  - Elemental oxygen
  - Photons (UV and other spectrum)

Image source: USDA - ARS

Back to Basics: States of matter



a plasma

ionized gas with random molecule movement

Eg. ice water
Temp < 0°C

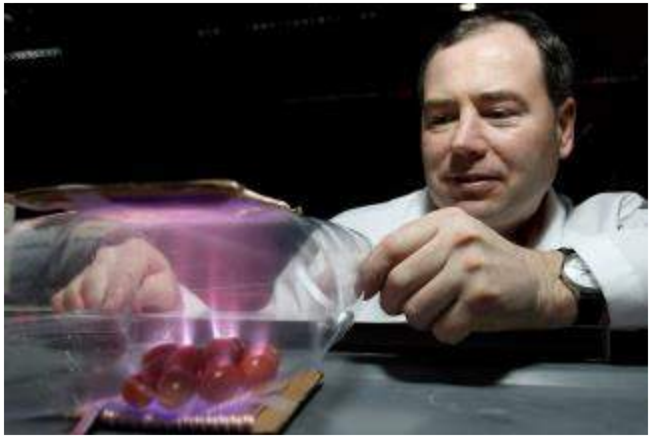
Eg. water
Temp 0 to 100 °C

Eg. steam
Temp > 100°C

Eg. ionized gas
$H_2 \rightarrow H^+ + H^+ + 2e^-$
Temp >100,000°C

In package sterilization

- Package is used as a dielectric barrier
  - Package becomes an insulator
  - Voltage passes through the package
- H_2O_2, ozone, nitrogen oxide, etc.
- Sterilizing fresh produce packaged in a 1 gallon bag ~ 0.10 ¢ for 50 to 100 W treatment
- Gliding arc non-thermal treatment of apples, cantaloupe and other fresh products (Brenden Niemira and group, USDA ARS)
- Apples, cantaloupe and lettuce
  - *Escherichia coli* O157:H7, *Salmonella spp.*, *Listeria monocytogenes*



Dr. Kevin Keener, Purdue

Source: Misra et al., 2011;

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Quality

- Very few studies done on the quality of cold plasma treated foods
- Germination of grains and legumes are preserved (Selcuk et al., 2008)
- Increased flavanols in lambs lettuce treated with cold plasma (Grzegorzewski et al., 2010)
  - No change in phenolic compounds such as caffeic acid and chlorogenic acid
- Strawberries
  - No change in color, firmness or respiration rate (Misra et al., 2014b)
- Blueberries (Lacombe et al. 2015)
  - Significant reduction in firmness for treatments >60 s
  - Significant reduction in anthocyanins after 90 s
  - Significant changes in surface color (> 120 s for L* [lightness/darkness]and a* values [red/green] and 45 s for b* values [yellow/blue])

Quality

- Cherry tomatoes treated with cold plasma

(a) (b)

After 14 days of storage at room temperature in a closed container (Source: Keener, Purdue University)

- Cherry tomatoes treated with cold plasma (Misra et al., 2014a)
  - No change in color, firmness, pH and weight loss

Future outlook and conclusion

- Cold plasma is a promising technology for surface decontamination of foods, packaging materials and biofilms
- Effect of cold plasma on sensitive food components (lipids, vitamins etc.) need to be better understood and optimized
- Inactivation mechanisms need to be better understood → what species are responsible for inactivation (how to ensure same species are generated each time – accounting for variability)
- Safety of feed gases need to be investigated

Future outlook and conclusion

- Regulatory
  - What is being measured and controlled → how to make this consistent?
  - Systematic studies and validation are needed → approval
- Significant amount of research is needed for food related applications
- Technology can be easily adapted and scalable based on applications in other fields

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Leveraging Technology to Improve Produce Safety and Quality

Are the costs worth it?

Perspectives on using genomic sequencing data

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Executive Director,

Alliance for Advanced Sanitation

ALLIANCE FOR ADVANCED SANITATION

Genetic sequencing and the food supply

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Production
Distribution
Processing
SUPERMARKET
Restaurant
Retail
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5 Approaches

- Routine Quality
- Regulatory Burden
- Investigatory Tool
- Competitive Edge
- Cost Reduction Method

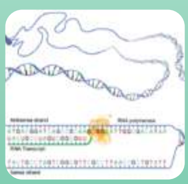
Genomics Provides Useful Tools for Manufacturing

Whole Genome Sequencing

Foodborne disease outbreak investigations

Metagenomics

Diversity of the entire sample/ecosystem



Transcriptomics

Specific target data and functionality

Many of these tools are currently being used to innovate in the area of enzymes, understanding the expression of genes and to better classify or identify microorganisms.

These technologies are currently utilized in microbiology labs to investigate spoilage issues, nutritional product development, and in understanding the human microbiome and gut microbiology that can be vital in how new product innovation strategies are developed.

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Genetic sequencing and the food supply

Use the right tool for the purpose

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Approaches to Study the Microbiota of Different Environments

Microbiota of a food sample

Question	Approach	Outcome
Who Is There?	Amplicon Metagenome: 16S rRNA	Bacterial Identification
What Can They Do?	• Metagenome: Whole Genome DNA Sequencing	•Bacterial identification •Insight into functional potential
What Are They Doing?	• Metatranscriptomics: RNA • Metaproteomics: Protein • Metabolomics: Metabolites	Inference of Functional Activities

Addis. F.M. *et al.*, The bovine milk microbiota: insight and perspectives from-omics studies. Mol. BioSyst., 2016, 12, 239-2372

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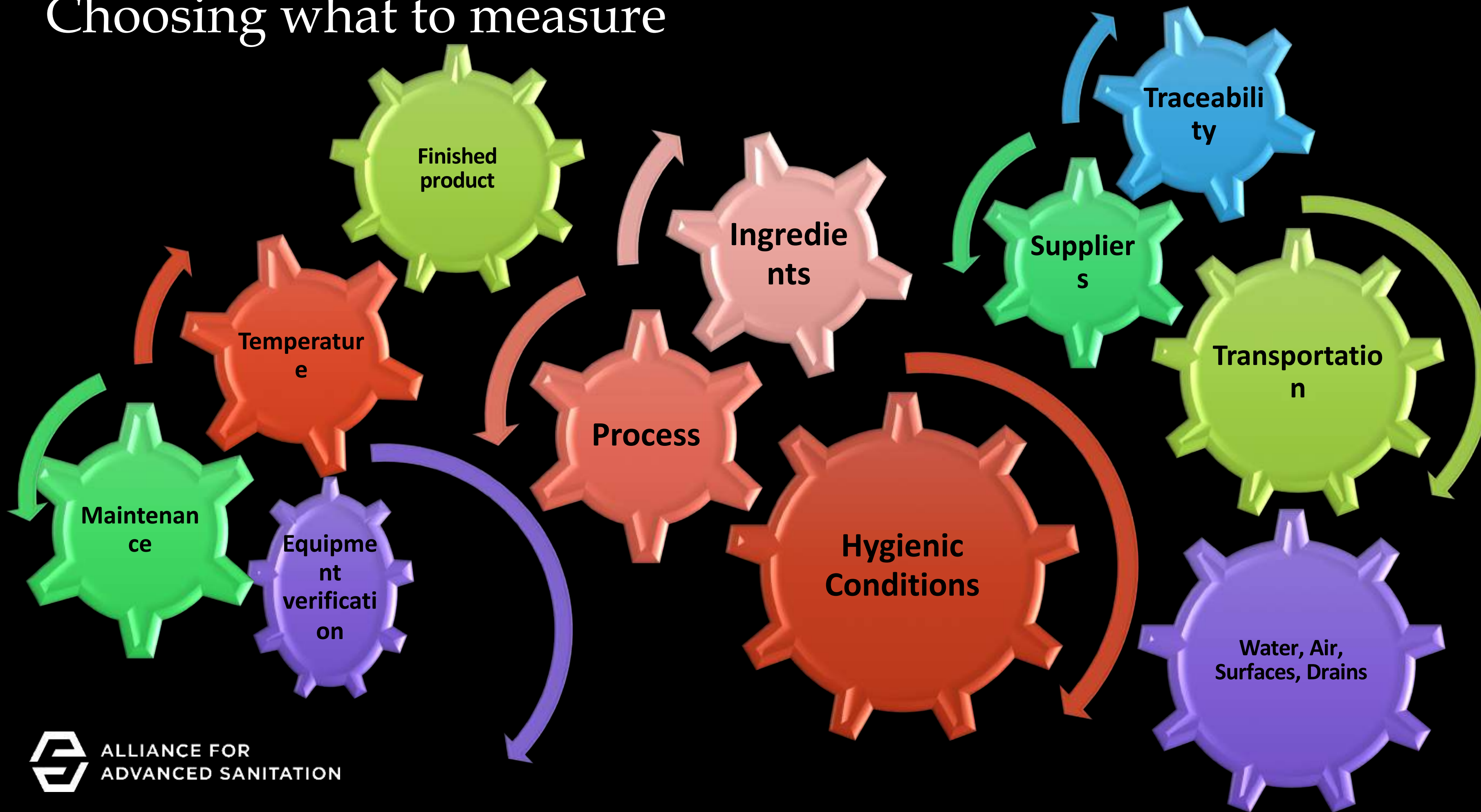
Choosing what to measure



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Market For Genetic Sequencing

Growth rate between 17-19%

Annually (CAGR)

Includes all types of sequencing

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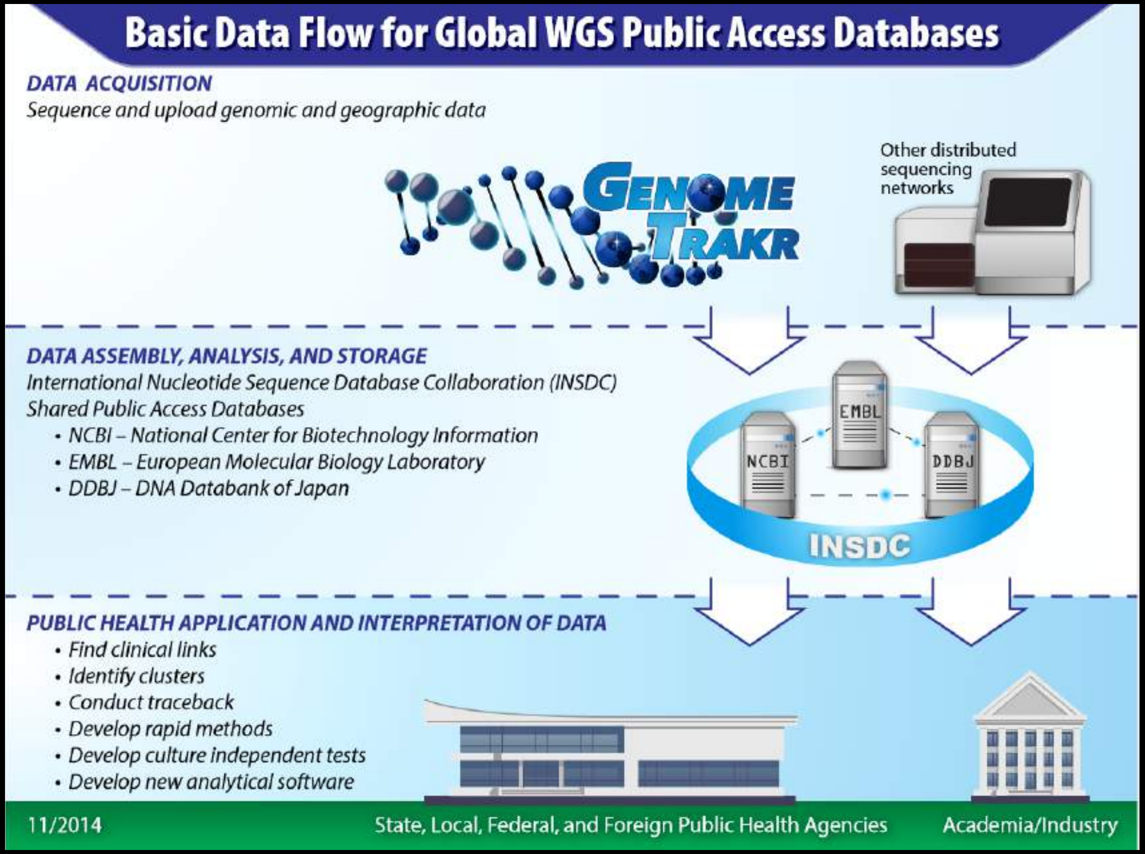
PRESS RELEASE

Next Generation Sequencing Market Size will reach 15 billion USD by 2025

Published: Apr 30, 2019 4:06 p.m. ET

Apr 30, 2019 (Heraldkeeper via COMTEX) -- The 'Next Generation Sequencing market' analytical summative by Market Study Report, LLC, is a thorough study on the latest market trends prevailing in the global business sphere. The report also offers important details pertaining to market share, market size, profit estimations, applications and statistics of this industry. The report further presents a detailed competitive analysis including growth strategies adopted by key players of the industry.

Next Generation Sequencing Market will exceed USD 15 billion by 2025; as per a new research report.



Basic Data Flow for Global WGS Public Access Databases
DATA ACQUISITION
Sequence and upload genomic and geographic data
GENOME TRAKR
Other distributed sequencing networks
DATA ASSEMBLY, ANALYSIS, AND STORAGE
International Nucleotide Sequence Database Collaboration (INSDC)
Shared Public Access Databases
• NCBI – National Center for Biotechnology Information
• EMBL – European Molecular Biology Laboratory
• DDBJ – DNA Databank of Japan
EMBL
NCBI
DDBJ
INSDC
PUBLIC HEALTH APPLICATION AND INTERPRETATION OF DATA
• Find clinical links
• Identify clusters
• Conduct traceback
• Develop rapid methods
• Develop culture independent tests
• Develop new analytical software
11/2014
State, Local, Federal, and Foreign Public Health Agencies
Academia/Industry

Risk determines priority

- Routine Quality
- Regulatory Burden
- Investigatory Tool
- Competitive Edge
- Cost Reduction Method