Protocatechuic Acid (PCA); a natural contamination preventative for the food industry.

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Title: Protocatechuic acid (PCA); a natural contamination preventative for the food industry.

Eligibility criteria is met for the **Small business Award.**

Focus Area 3: Design of greener chemicals. Protocatechuic acid (PCA) is a phenolic acid, a green chemical reagent, presently categorized as biocide by EPA. PCA is native to the ecosystem, everywhere in nature; soil, water ways, all plants, fruit and vegetables. PCA is common to the human diet. The bacteria in the human bowel manufacture a small amount daily. It is designated at G.R.A.S. by FDA. PCA is safe by every measure. It is non-toxic, non-allergenic and non-mutagenic.

The Need: The need in well recognized. For example, a recent economic and societal example was the multi-million-dollar loss for the lettuce industry in 2018 and 2019. https://www.cdc.gov/ecoli/2018/o157h7-11-18/index.html

The bacterial pathogens are common and many; i.e. E. coli, Pseudomonas and Salmonella. Further complicating the solution is that the location of the fomites is multifactorial prior to the consumer; the field, the plant, the harvesters, the harvesting method, the assembly lines and workers, the packaging and the workers, shipping and shippers and market handlers and displays. Today there is an urgent need to find new ways to provide control of food produce contamination and safety. The solution is multifactorial as well built around a center piece of a natural anti-microbial reagent, PCA.

Most recent Milestones are the critical discoveries resulting in a US patents application using protocatechuic acid (PCA) in the food industry. The work is original and novel as evidenced by the US patent US 10,292,946; May 21, 2019 @ https://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=10,292,946.PN.&OS=PN/10,292,946&RS=PN/10,292,946.

Publication: Clinical testing on human skin showed the control of pathogens, while preserving commensal bacteria. *Jalali, Omid; Best, Molly; Wong, Alison; Schaeffer, Brett; Bauer, Brendon; Johnson, Lanny. Reduced Bacterial Burden of the Skin Surrounding the Shoulder Joint Following Topical Protocatechuic Acid Application Results of a Pilot Study. JBJS Open Access d 2020:e19.00078. http://dx.doi.org/10.2106/JBJS.OA.19.00078*

It is known that protocatechuic acid is an anti-microbial by topical application in various applications. *US patents 10,004,705, 10,004, 706, 10,398,664, 10,426,747, 10,772, 860, 10,959,969, and 11, 103, 471.*

The US Component: The inventor is an US citizen. All the research was performed at independent contract laboratories in USA. The assembly of the commercial product is at all independent contact USA GMP facilities.

Abstract:

This Green Chemistry Challenge submission is one of using a natural reagent, PCA as an anti-microbial in the food industry; from harvesting, via production and distribution to consumption.

PCA is a phenolic acid, a biocide by EPA designation. Importantly for this application, PCA is safe by every measure. The human pharmacokinetics and pharmacodynamics are known.

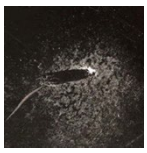
PCA in food production and distribution is intended to introduce a natural antimicrobial solution where there are only toxic chemical alternatives. Observations of the supply chain from harvesting to consumer showed additional need for implementation of new sanitary procedures.

The implementation is eminent, only awaiting EPA approval. The raw material is available in 1000-kilogram quantities. There are multiple registered manufacturing facilities in USA for producing a spray containing PCA. Distribution channels are in place and a known market waiting for purchase.

The quantitative benefit to the environment is the use of a chemical native to the environment. The amount of the alternative toxic reagent to be replaced is not known. The implementation is non-existent, diverse, varied and localized. PCA use in miniscule amounts would provide a safe and effective method of controlling contamination in the food industry.

The experimental evidence supporting the US patent application above, showed a PCA spray of as little as 0.10-1.25% leaves an invisible lasting protective crystalline coating on an article; face mask, plastic or metal. The bacteria including biofilms are killed upon contact. The encoated virus, SARS CoV-2 is likewise inactivated; US patent 10, 959, 969.

The vehicle may be water and or alcohol, both being safe when consideration is given to the specific use and the target article. Upon delivery to a hard surface fomite, the vehicle evaporates leaving a uniform, invisible coalescence of PCA crystals adhered to the intended surface as illustrated in the following photograph.



For illustration purposes, excess concentrated PCA coating was purposely applied to a filing cabinet. Two years later, the crystals were scraped with the fingernail. Note the 2x4 mm area, not to scale and pile of crystals to the right.

On site observation of the food industry practices indicates that many new procedures at various points of contact are necessary. The harvester and the production worker would use the PCA spray on exposed hands and gloves. Clinical type face masks and protective personal equipment uniforms should be instituted and sprayed. The harvesting hand equipment or knives should be cleaned, sprayed and interchanged regularly. The assembly line should be sprayed to have a lasting uniform protective coating of the PCA crystals. Packaging and the handlers should be subject to protective spraying as well as shippers and vehicles.

It would be safe to have a low dose of 0.10% PCA in aqueous vehicle on various produce themselves, including the misting sprays seen continuously on super market produce shelves.

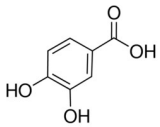
The multiple locations of PCA use and disposal would be wide spread and the resulting amounts would be miniscule. Water way pollution would not exist because the PCA source is not a result of food production.

Scope of the program

The use of protocatechuic acid (PCA) in the food industry has real benefits when instituted from harvesting to consumer.

The Dose: The dosage terminology requires some clarification. PCA in an aqueous delivery vehicle would be 0.10 to 1.24%, the later the maximum solubility in water. The concentration in alcohol need only be 1% and rapidly evaporate. However, the resultant lasting PCA crystalline coating on the article would be 100%.

Chemical Nature: PCA is a phenolic acid; 2,4 di-hydroxybenzaldehyde. The CAS number is 99 50 3. The raw crystal is white to tan in color and 80 mesh in size.



Realized Benefits and Drawbacks

Benefits: The use of PCA accompanying by implementation of better harvesting, handling and distribution procedures would decrease the opportunity for contamination in the food industry.

PCA has been recognized as safe for ingestion by FDA as a food flavoring additive. PCA has an existing FDA G.R.A.S. designation as Generally Recognized As Safe.
http://www.ift.org/~/media/Food%20Technology/pdf/2009/06/0609feat_GRAS24text.pdf
PCA is non-toxic, non-allergenic and non-mutagenic. It is not inflammable.

PCA's presence in this application reduces the opportunity for contamination in produce, fruits and vegetables. As show in the patents above, a PCA crystalline coating is an anti-bacterial, biofilm destroying and anti-viral for encoated virus; i.e. SARS-CoV-2 when applied to an article of hard or cloth surface. PCA is a disinfectant on human skin in as little as 1% PCA in water as reported in the publication noted above.

This addition PCA to the food industry would have no adverse effect upon the fruits and vegetables in which PCA already exists in small amounts. The thin coating on fruits and vegetables would be added protection from the contamination of multiple customers handling.

There is safety for the environment since is already has an ecological presence.

The amount of PCA introduced to the environment in this manner would have minimal effect upon the existing ecology. The amount would be small and dispersed widely to many locations. When used in the intended manner, PCA is further dispersed from it point of origin along the supply change to the purchaser. The PCA will be returned in small amounts to the environment as sewage; septic field and or community sewage plants in a multitude of locations. The end result will be a miniscule amount of PCA in any given location, including the produce point of

origin due to the subsequent dispersion. A minimal amount would be added to the already abundant amount in the environment. PCA in this application will be well dispersed via the natural distribution and will not be a pollutant, but only add small amount to the existing PCA ecological presence. The use of PCA will not produce any carbon dioxide nor require any extra use of energy.

Drawbacks: The real and or potential drawbacks are not known. There may be a small concentration of PCA at the site of production, but the crystals are adherent to the articles; clothes, masks, machinery, containers, etc. The use at the sites of origin would have minimal effect on the local water ways. PCA's potential toxicity to the waste water is unlikely due to minimal discharge from the fomite. Das, et al concern for low amounts of PCA being toxic is refuted by studies showing same amounts to be anti-bacterial and causing accelerated healing of mammalian wounds.

The potential inflammability is nil with the dry crystalline residue. The same with water. It would have transient inflammability in alcohol until evaporation. There are no fumes from the PCA crystal.

Pilot Plant Exists: The reagent PCA is available from many international sources. There are many US GMP plants available for manufacture into a commercial product.

Manufacture: The PCA reagent is presently biochemically manufactured, thereby eliminating the trace metals found in previous production by extraction from plant material. Certificate of Analysis accompanies each production which is subsequently verified by independent laboratory for percentage and purity.

EPA application initiated in Jan 2020. Still under review.

Technology Comparisons: PCA is not found in other disinfectants. PCA is native to the environment. It is safe by any measure. Although prices are rising due to shipping costs plus world-wide inflation the cost of goods for PCA is relatively low, such to be commercially viable and competitive with the existing market.

Performance has been established in proof of principle pilot studies supporting US patents and published.

Planned Commercialization: Commercialization awaits an EPA application that was submitted 23 months prior to this writing. The reagent is available in 1000-kilogram quantities. The manufacturing facilities are in place.

Technology has not been launched.

The Commercial Products: PCA is delivered as raw crystal in various size sealed containers. The shelf life is a minimum of 2 years.

Application: Delivery as aqueous and or alcohol spay containers.

Science and Innovation: The application is original, and determined novel as evidence by the multiple US patents for various applications. This is further evidenced by the US patent 10, 969, 959 was issued 7-month after submission of application, because there was no prior art.

There scientific publications of use on human hands is noted above.

Human Metabolism: Should PCA be ingested, the pharmacokinetics and pharmacodynamics are known; already in medical literature.

Mode of action: The anti-bacteria and anti-viral modes of action are well known in the literature and by evidence supporting US patents.

Bacterial Mode of Action: Inhibitory mechanisms of PCA on bacteria growth are wide-ranging and include

- destabilizing the bacteria cytoplasmic membrane,
- altering the permeability of the bacteria plasma membrane,
- inhibiting extracellular microbial enzymes,
- directly altering microbial metabolism, and
- depriving microbes of substrates required for growth [1].

Specifically, PCA can;

- change bacterial physicochemical surface properties. For example, ferulic acid has been shown to decrease hydrophobicity of Pseudomonas aeruginosa [2]. PA treatment can also
- alter bacterial polarity by changing bacteria surface electron acceptors on both gram-positive (increased acceptor components) and gram-negative (decreased acceptor components) strains [2].
- It has been shown that as PA concentrations increase, the percentage of cell membrane damage significantly increases, as indicated by release of intracellular K+, with greater effects observed with gram-negative bacteria strains than with gram-positive bacteria strains [2]

1: Dietrich, H.; Pour Nikfardjam, M.S. Influence of Phenolic Compounds and Tannins on Wine-Related Microorganisms. In Biology of Microorganisms on Grapes, in Must and in Wine; König, H., Unden, G., Fröhlich, J., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 421–454, ISBN 978-3-319-60021-5.

2. Borges, A.; Ferreira, C.; Saavedra, M.J.; Simões, M. Antibacterial Activity and Mode of Action of Ferulic and Gallic Acids Against Pathogenic Bacteria. Microb. Drug Resist. 2013, 19, 256–265.

Anti-Bacterial Duration of Action: The duration of action of PCA crystalline coated metal and polyester was a minimum of 48 hours with an unprecedented 99.999% destruction MRSA and Pseudomonas biofilms.

PCA'S Anti-Viral Mode of Action is the **Host Targeting Principle**: The PCA crystal by its inherent sharp physical structure (see above) physically disrupts the surface of the bacteria or an encapsulated virus upon contact. PCA therapeutic effect is initiated by physical disruption of the

virus coating. This is in contrast to other non-physical biological methods for capsular disruption.

Importance of the physical disruption of the viral coating in Host Targeting: Jackman and Cho described the lipid coating as the "common denominator" of all enveloped viruses; a group that includes flaviviruses, alphaviruses, coronaviruses, filoviruses, retroviruses and more. No other shared feature exists broadly across all those diverse viruses, which is why he proposes host-targeted antivirals might have greater potential as pandemic-preparedness tools.

Jackman JA, Shi P-Y, Cho N-J. Targeting the Achilles Heel of Mosquito-Borne Viruses for Antiviral Therapy. ACS Infectious Diseases 2019 5 (1), 4-8DOI: 10.1021/acsinfecdis.8b00286

Following the physical disruption, the other antimicrobial properties of PCA become active.

The crystal structure of the reagent causes physical disruption of bacterial and viral coating resulting in bacteria death and viral inactivation.

Biological Disruption: The medical literature substantiates the following known antiviral properties are found in PCA. These take effect in addition to the physical disruption.

- low pH; p*K*a of 4.49.

Dawson, R. M. C.; et al. (1959). Data for Biochemical Research. Oxford: Clarendon Press.

- anti-protease
- anti-docking reagent or docking inhibitor.

Elsbaey, et al report included that PCA bound well to multiple targets for SARS CoV-2 virus, including Mpro and PLpro.

Elsbaey M, Ibrahim MAA, Bar FA, Elgazar AA. Chemical constituents from coconut waste and their in silico evaluation as potential antiviral agents against SARS-CoV-2. S Afr J Bot. 2021 Sep;141:278-289. doi: 10.1016/j.sajb.2021.05.018. Epub 2021 May 28. PMID: 34092840; PMCID: PMC8162769.

Inhibitor of Mpro.

> Acar reported "PCA to have a binding affinity of -4.9 kcal/mol and effective against Mpro. The docked pose of protocatechuic acid with main protease (7BQY) and ligand interaction of protocatechuic acid with 7BQY." PCA had a Log*P* of 0.88 which means it was easily absorbed. With a positive CYP34, PCA would have capacity to cross the blood brain barrier.

Acar A. Evaluation of phenolic acids of Corylus avellane L. as a potential SARS CoV-2 Main protease inhibitors. Erzincan University. Journal of Science and Technology. 2021, 14 (2), 492-509. DOI: 10.18185/erzifbed.897348.

- cellular and hormonal immunity.

Guo, et al concluded that PCA effect was due to enhanced cellular and hormonal immunity.

Guo Y, Zhang Q, Zuo Z, et al. Protocatechuic acid (PCA) induced a better antiviral effect by immune enhancement in SPF chickens. Microb Pathog. 2018; 114:233-238. doi: 10.1016/j.micpath.2017.11.068.

Anti-Viral Duration of Action: The independent contract laboratory in vitro testing at MRIGlobal and Illinois Institute of Technology showed a PCA crystalline coating on an article of metal, plastic and material from N95 mask inactivated the SARS CoV-2 virus upon contact at 10 and 60 minute and at minimum of 24 hours following dry crystalline coating on the article.