Pulsed Light and Pulsed Electric Field for Foods and Eggs

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ABSTRACT

Two new technologies for use in the food industry are described. The first method discussed uses intense pulses of light. This pulsed light (PureBright®) process uses short duration flashes of broad spectrum "white" light to kill all exposed microorganisms, including vegetative bacteria, microbial and fungal spores, viruses, and protozoan oocysts. Each pulse, or flash, of light lasts only a few hundred millionths of a second (i.e., a few hundred microseconds). The intensity of each flash of light is about 20,000 times the intensity of sunlight at the earth's surface. The flashes are typically applied at a rate of about one to tens of flashes per second. For most applications, a few flashes applied in a fraction of a second provide an effective treatment. High microbial kill can be achieved, for example, on the surfaces of packaging materials, on packaging and processing equipment, foods, and medical devices as well as on many other surfaces. In addition, some bulk materials such as water and air that allow penetration of the light can be sterilized. The results of tests to measure the effects of pulsed light on Salmonella enteritidis on eggs are presented.

The second method discussed uses multiple, short duration, high intensity electric field pulses to kill vegetative microorganisms in pumpable products. This pulsed electric field (or CoolPure™) process can be applied at modest temperatures at which no appreciable thermal damage occurs and the original taste, color, texture, and functionality of products can be retained.

(Key words: pulsed light, pulsed electric field, microbial kill, cool pasteurization, egg)

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INTRODUCTION

PurePulse Technologies has developed two new processes for killing microorganisms associated with packaging, food products, medical supplies, pharmaceuticals, water, and air. These new processes use intense pulses of light or electric fields.

The pulse light (PureBright®) process uses short duration flashes of broad spectrum "white" light to kill a wide range of microorganisms, including microbial and fungal spores. Each pulse or flash of light lasts only a few hundred millionths of a second (i.e., a few hundred microseconds). During each flash, the intensity of the light is about 20,000 times the intensity of sunlight at the earth's surface. The flashes are typically applied at a rate of about 1 to 20 flashes per second. For most applications, a few flashes applied in a fraction of a second provide an effective treatment. High microbial kill can be achieved, for example, on the surfaces of packaging materials, on packaging and processing equipment, foods, and medical devices as well as on many other surfaces. In addition, some bulk materials like water and air that allow penetration of the light can be sterilized.

The pulsed electric field (CoolPure™) process uses multiple, short duration, high intensity electric field pulses to kill vegetative microorganisms in pumpable products. Because the process can be applied at modest temperatures at which no appreciable thermal damage occurs, the original taste, color, texture, and functionality of products can be retained.

PULSED ENERGY PROCESSING

Both of these new technologies, PureBright® and CoolPure™, use a technique known as pulsed energy processing. By storing electrical energy in a high energy density electrical storage capacitor and releasing it in short, high intensity pulses, high peak power levels can be generated. Such high peak power pulses of electrical energy can be used to create intense pulses of light or pulses of high electric field. The high intensity of these pulses results in unique bactericidal effects that are not observed when the same energy is provided at low intensity in a sustained or continuous wave (CW) mode. The enhanced bactericidal effects of pulsed light and pulsed electric fields provide a basis for new, highly effective processes for sterilizing, or reducing bacterial contamination associated with many different products.

Although the peak power of each pulse is very high because of the short duration, the total energy in each pulse is relatively low, and the average power require-
broadband "white" light. The emitted light pulse has
ran), through the near UV (300 to 380 nm) and visible
processes, in addition to being effective, are economical.

**PULSED LIGHT (PureBright®)**

The PureBright® light pulse is generated by electrically
ionizing a xenon gas lamp, causing it to emit a
broadband “white” light. The emitted light pulse has
wavelengths from the far ultraviolet (UV) (200 to 300
nm), through the near UV (300 to 380 nm) and visible
(380 to 780 nm), to the infrared (780 to 1,100 nm).
Approximately 25% of the light is UV, 45% visible, and
30% infrared. The duration of the light pulse is typically
200 to 300 µs, and lamps are generally flashed from one
to tens of times per second. Because only one to a few
flashes are required to provide high microbial kill levels,
the process is very rapid and amenable to high
throughput.

The light is nonionizing and does not penetrate
opaque materials, but is transmitted through many
packaging materials and therefore may be used to treat
packaged products. The primary effects of treatment,
and the main antimicrobial mechanisms, relate to the
rich content of broad spectrum UV light used; and the
very high intensity, and short duration nature of the
treatment. PureBright® treatment of microorganisms
suspended in 25 µL droplets of water and dried onto the
surface of bacteriological media in a Petri dish produces
very high kill levels, and the results indicate the kind of
killing effects commonly obtained on smooth surfaces
and packaging materials. A PureBright® intensity of 1.5
J/cm² kills > 7 logs/cm² of Staphylococcus aureus; and >
7 logs/cm² of Bacillus subtilis spores are killed using 4 J/
² cm² in Petri dish tests. (One Joule of energy is
approximately a quarter of a calorie, i.e., about 4 J of
energy deposited in 1 g of water will raise its
temperature 1 C. Also, 1 W is equivalent to 1 J/s;
therefore a 100-W light bulb would operate for 1/100th
of a second on 1 J of energy.) Tests have been performed
to measure the killing effects against a wide range of
microorganisms; all microorganisms tested, including
vegetative bacteria, fungal or bacterial spores, protozoan
oocysts, viruses, etc., are killed efficiently by PureBright®
treatment.

When compared to conventional UV light from a CW,
high intensity mercury vapor lamp [such as a germicidal
lamp or a UV-C lamp (Brown Boveri)], PureBright® kill
dynamics are significantly better. Using relatively resis-
tant Aspergillus niger spores dried on a packaging
surface, conventional CW UV kills ~ 3.5 to 4.5 logs in a 6
to 10 s period. Longer exposure times do not signifi-
cantly increase the level of CW UV kill obtained. By
comparison, PureBright® kills > 7 logs/cm² of these A.
niger spores in one to two flashes in a fraction of a
second, thereby providing a much higher kill level in a
much shorter treatment time.

PureBright® can kill high levels of microorganisms on
packaging surfaces. The same high levels of kill are also
realized on equipment, instrument, and device surfaces.
Because the light is readily transmitted through water
and air, high microbial kill levels can also be attained in
these media.

Due to the generally opaque and irregular surface of
foods, lower kill levels are produced. However, signifi-
cant shelf life extension of perishable foods can be
achieved and risk of pathogenic microorganisms can be
reduced.

**Packaging**

PureBright® can be used to sterilize packaging material
surfaces for the food, medical, and pharmaceutical
industries. The process can be easily designed into
packaging lines. The PureBright® treatment time is
generally shorter than times required for other line
processes, so the treatment is not a rate limiting factor.
Each pulse of light is easily monitored through fiber optics
and feedback circuits to assure that proper treatment is
provided, so the process is essentially fail-safe. Bacterial
spore kill has been demonstrated at levels high enough for
aseptic packaging use and full scale commercial testing is
underway.

**Foods**

PureBright® can be used to reduce microbial loads for
fresh fruits and vegetables, baked goods, fresh and
processed meat, poultry and fish, eggs, certain liquids,
and free flowing particulates. The benefits include
reducing public health risk from foodborne surface
pathogens, extending shelf life of perishable foods, and
improving yields or economics during perishable foods
distribution.

Extended shelf life of treated fruits and vegetables has
been demonstrated. Refrigerated tomatoes have been held
satisfactorily for 36 d whereas the untreated controls show
spoilage. The same general effect is true for plums, pears,
strawberries, and raspberries. For certain produce,
PureBright® inhibits enzymatic activity, retarding surface
discoloration and delaying softening and subsequent
microbial attack.

Significant shelf life extension of baked goods can be
realized by the elimination of mold spores from surfaces.
In one case, the shelf life of bread sticks was increased
from 6 to 20 d.

The level of microorganisms on meat surfaces can be
reduced 1 to 3 logs by PureBright®, thus reducing the risk
of pathogens and extending refrigerated shelf life by
several days. Escherichia coli on beef, Salmonella on chicken
wings, and Listeria on hot dog wieners have all been
reduced 1 to 3 logs.

PureBright® can also be used to treat perishable foods
through plastic films, thus avoiding any recontamination
of the product.

**Eggs**

Tests were performed to determine the microbiological
effect of PureBright® treatment on eggs. Two sources of
eggs were used for testing: commercial eggs off-the-shelf from a local San Diego grocer, and raw unwashed eggs from a local farm.

Eggs were inoculated by immersion for 10 min in a saline-peptone water (0.9% saline, 0.1% peptone) suspension of an overnight tryptic soy broth shake culture of *S. enteritidis* (ATCC 13076) followed by a brief drip drying under ambient conditions. In one set of tests the recovery from control (untreated) and PureBright®-treated eggs was compared using a wash-in-bag recovery method. In this method, each egg was shaken vigorously (but without breakage) in a volume of saline-peptone water. The wash suspension was then enumerated using standard methods without plates incubated 48 h at 30 °C. The inoculation and recovery procedures used were designed to primarily test the effects of PureBright® treatment against microorganisms on the surface of eggs. The results comparing microorganism recovery from control (untreated) and PureBright®-treated eggs are shown in Table 1.

The PureBright®-treated samples were flashlamp pulsed with eight flashes at 0.5 J/cm² per flash (for a total treatment of 4 J/cm²). These results show that PureBright® is very effective in eliminating microbial contamination from the surface of shell eggs. As much as 8 logs of *S. enteritidis* kill was obtained. No difference was noted between commercial and unprocessed eggs.

In a second set of tests, eggs were inoculated and PureBright®-treated as before, except the temperatures of the eggs and the inoculum solution were carefully controlled to measure the effects of temperature difference during inoculation. It is well known that under some conditions of temperature differential, inoculum is drawn deep into the pores of the egg (even to the level of the egg membrane) by an aspiration effect within the egg. After treatment, the whole eggs (including the shells) were liquified in saline solution by either stomaching or blending. For obvious reasons, the wash-in-bag assay was not used for microorganism recoveries here. Instead two methods of recovery were tested: stomacher digestion of the egg with an added volume of recovery medium (saline-peptone), and mixing recovery medium and egg in a cooled Waring blender. The resulting mixture was then plated. The results are shown in Table 2. It is clear that temperature difference between the egg and the inoculum influences the ability of PureBright® to reduce microbial counts on eggs. The instance in which there is a positive temperature difference (inoculum is colder than the egg) promotes aspiration of the inoculum into the inner egg through pores in the shell. In this instance, the response to treatment can be used to test the ability of PureBright® to kill organisms not just on the surface of eggs, but also in the egg pore. Significant reductions in recovery between control and treated eggs were still observed. This result strongly suggests that the PureBright® killing effect on shell eggs is not limited to surface microorganisms, but also extends to a degree into the egg pore.

### Medical-Pharmaceutical

Numerous potential applications for PureBright® exist in these industries. These applications include, but are not limited to, treatment of packaging materials, treatment of prepackaged fluids that transmit UV light, treatment of medical devices and instruments, treatment of equipment surfaces, and treatment of air or water for hospital, clean rooms, and other environments.

### Water Treatment

High levels of kill of bacterial spores, *Cryptosporidium* oocysts, *Klebsiella terragenes*, and viruses have been demonstrated in water. Applications being investigated include treatment of municipal water; waste water; bottlers make-up water; and packaged drinking water before, during, or after packaging.

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**TABLE 1. Effect of PureBright® on Salmonella enteritidis on unprocessed and commercial shell eggs**

<table>
<thead>
<tr>
<th>Control recovery</th>
<th>Treated recovery</th>
<th>Log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>(logs)</td>
<td>(0.5 J/cm²/flash, 8 flashes)</td>
<td>(logs)</td>
</tr>
<tr>
<td>Unprocessed eggs: Wash-in-bag recovery</td>
<td></td>
<td></td>
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<tr>
<td>3.90</td>
<td>0</td>
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<tr>
<td>7.88</td>
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<tr>
<td>7.97</td>
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</tr>
<tr>
<td>7.92</td>
<td>0</td>
<td>&gt;7.92</td>
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<tr>
<td>Commercial eggs: Wash-in-bag recovery</td>
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<td></td>
</tr>
<tr>
<td>3.66</td>
<td>0</td>
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<tr>
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</tr>
<tr>
<td>6.78</td>
<td>0</td>
<td>&gt;6.78</td>
</tr>
</tbody>
</table>

1Very high kill of surface inoculum.

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**TABLE 2. Influence of temperature difference between egg and inoculum on PureBright® kill on shell eggs**

<table>
<thead>
<tr>
<th>Temperature difference (C)</th>
<th>Control recovery (logs)</th>
<th>Treated recovery (logs)</th>
<th>Log reduction</th>
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<tbody>
<tr>
<td>2</td>
<td>3.90</td>
<td>0</td>
<td>&gt;3.90</td>
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<tr>
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<td>5.50</td>
<td>0</td>
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<td>7.59</td>
<td>3.55</td>
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<tr>
<td>27</td>
<td>7.84</td>
<td>5.62</td>
<td>2.22</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature difference (C)</th>
<th>Control recovery (logs)</th>
<th>Treated recovery (logs)</th>
<th>Log reduction</th>
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<td>&gt;3.71</td>
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1Temperature difference = egg (C) – inoculum (C). Negative values mean that the inoculum is warmer than the egg. Positive values mean that the inoculum is colder than the egg.
2Eggs mixed in stomacher.
3Eggs mixed in Waring blender.
**Regulatory Factors**

A petition was submitted to the FDA in February 1994 for approval of PureBright® treatment of foods.

**Economics**

The economics of the PureBright® process are very attractive. A conservative estimate of PureBright® treatment cost at 4 J/cm², including equipment amortization, lamps, electricity and maintenance, is 0.1¢/ft² of treated area.

**PULSED ELECTRIC FIELDS (CoolPure™)**

The CoolPure™ pulsed electric field process involves treating a pumpable product with a series of short duration, high intensity electric field pulses. The pulses cause electroporation of the cell wall of vegetative microorganisms such as bacteria, yeasts, and molds. Under proper treatment conditions, the poration is so massive that the cell cannot recover and dies.

The treatment chamber consists of two electrochemically inert electrodes between which the product passes. High intensity pulses of electric field are applied between the two electrodes. The electric field pulses are 1 to 10 µs in duration and electric field strengths of 20 to 80 KV/cm are typically used to treat product flowing through the treatment chamber. The product residence time within the chamber, the pulse repetition rate or frequency, and the duration, intensity, and shape of the pulse determine the level of treatment. The electric field pulses lead to the generation of some heat in the product, but the treatment parameters can be controlled to optimize kill without exceeding the thermal damage threshold of the product.

The nature and level of energy transfer is such that no significant chemical changes occur in the product. This is supported by theoretical calculations as well as in-depth testing and analyses of treated foods such as milk and fruit juice that show no change in product chemistry.

A 200 L/h continuous flow CoolPure™ Laboratory Pilot System is in operation at PurePulse Technologies in San Diego, CA. The system includes heat exchangers and temperature controls to regulate product inlet temperatures and to cool product after treatment. Process performance is continuously monitored to assure uniform product treatment.

CoolPure™ processing can provide the unique benefit of low temperature pasteurization for a large number of foods and food ingredients. Some of the benefits include: better retention of flavor, color, and nutritional properties; improved protein functionalities; eliminating damage to emulsions; increase in shelf life; reduced pathogen levels; and better control of fermentations.

**Milk**

Effective treatment of raw milk has been accomplished at 55 C (131 F) with bacterial kill levels in excess of those obtained with conventional time-temperature pasteurization conditions. In tests with inoculated Listeria innocua as a surrogate for Listeria monocytogenes, > 6 logs were killed using only a few seconds exposure at 55 C. Because milk treated with CoolPure™ has suffered less flavor degradation, it may be possible to manufacture dairy products such as cheeses, butter, and ice cream with improved flavor.

Extensive chemical analysis revealed no change in the chemical or physical properties of treated milk. Analyses included enzyme activity, fat integrity, starter growth, rennet clotting yield, cheese production, calcium distribution, casein structure and protein integrity.

**Liquid Eggs**

High levels of microbial kill can be achieved with CoolPure™, thus providing a safe product with extended shelf life.

**Juices**

Superior tasting fresh juices can be processed with lower bacterial counts than conventional pasteurization. Treated orange juice, for example, tastes like freshly squeezed juice, but has no microorganisms. Secondary thermal pasteurization of juices can be avoided by CoolPure™ treatment. Hot fill of formulated fruit juices and drinks can be avoided through the use of CoolPure™ treatment in conjunction with PureBright® treatment of the packaging material and aseptic filling.

**Emulsions**

Heat-sensitive emulsion products such as salad dressings can be pasteurized without breaking down the emulsion integrity.

**Food Ingredients**

Flavors, protein concentrates, and fermentation products can all be pasteurized more gently with CoolPure™, allowing retention of the quality parameters that permit superior quality.

**Regulatory Factors**

The potential for generating electrochemical changes in foods is insignificant. Documentation showing no potential for chemical change has been submitted to the FDA. The FDA has issued a letter of no objection to PurePulse Technologies for the use of CoolPure™ for the treatment of foods.

**Economics**

A conservative estimate of CoolPure™ treatment costs at 100 J/L, including equipment amortization, electricity, and maintenance, is less than 0.4¢/L.