A METHOD OF INACTIVATING A CONTAMINANT IN A BIRD'S NEST OR A PART THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to a method of inactivating a contaminant in a bird's nest or a part thereof.

BACKGROUND OF THE INVENTION

[0002] Edible bird's nest is a delicacy in Chinese cuisine and is usually consumed as bird's nest soup for nutrition and health purposes. It is widely believed by the Chinese community that the consumption of bird's nests provide health related benefits such as improved digestion problems, concentration, immune deficiencies, aphrodisiac properties and respiratory related conditions. Therefore, bird's nests are considered to be the one of the most expensive food products consumed by humans and are a lucrative trade in Asia.

[0003] Bird's nests are derived from Cave Swiftlets which can be found in parts of Asia such as Malaysia, Thailand, Vietnam and Indonesia. During the breeding season, the salivary glands of the swiftlet produce a thick and sticky saliva so that a nest cement is constructed in which eggs are laid. The bird's nests dry up quickly and are shaped like a half-moon cup stuck to the vertical cave wall. These bird's nests are then harvested from the caves and are highly sought after for use and consumption in Chinese cuisines.

[0004] Due to the emergence of potentially dangerous diseases such as SARS coronaviruses, and in particularly bird flu caused by the H5N1 virus, health authorities as well as consumers are more vigilant on the safety and hygiene of avian related food products including bird's nests.

[0005] Accordingly, it is an object of the present invention to provide a method of inactivating these contaminants in bird's nests that overcomes at least some of these disadvantages. In order to improve the safety and market value of bird's nests, it is vital to have bird nests products free from contaminants so that they will be safe and suitable for human consumption.

SUMMARY OF THE INVENTION

[0006] According to a first aspect, there is provided a method of inactivating a contaminant in a bird's nest or a part thereof, the method comprising subjecting the bird's nest or the part thereof to ultrasound. The method further includes an inactivation pre-treatment. The pre-treatment includes subjecting the bird's nest or the part thereof to UV radiation and/or exposing the bird's nest or the part thereof to ozone.

[0007] According to a second aspect, there is provided a method of inactivating a contaminant in a bird's nest or a part thereof, the method comprising: i) subjecting the bird's nest or the part thereof to UV radiation and/or exposing the bird's nest or the part thereof to ozone; and ii) subjecting the bird's nest or the part thereof to ultrasound.

[0008] In this context, it is noted that the inventor has found that the present invention has numerous advantages. While being effective to remove contamination, the method of the present invention has no implications on the taste, appearance and consistency on the treated bird's nest. Thus, the bird's nests that have been treated by the present invention meet customers' satisfaction and are safe for consumption.

DETAILED DESCRIPTION OF THE INVENTION

[0009] A method according to the invention is provided to inactivate a contaminant in a bird's nest or a part thereof. Edible bird's nests are derived from birds called Cave Swiftlets of the genus *Aerodramus*. *Aerodramus* comprises around 30 species with a habitat range covering southern Asia, south pacific islands and north eastern Australia located within the tropical and sub-tropical regions. Out of the 30 species, four species comprising *Aerodramus unicolor, Aerodramus fuciphagus, Aerodramus maximus and Aerodramus germani* have the highest commercial value. *Aerodramus unicolor* and *Aerodramus fuciphagus* produce high grade bird's nests while *Aerodramus maximus* produces lower grade black nests with more feathers, particles and other impurities. Since bird's nests may be considered to be avian related products, there is likelihood that contaminants such as avian influenza viruses may exist in bird's nests.

[0010] Therefore, in one embodiment, there is provided a method of inactivating a contaminant in a bird's nest or a part thereof, the method comprising subjecting the bird's

nest or the part thereof to ultrasound. The bird's nest or the part thereof may be placed in a liquid medium during ultrasound treatment. In one embodiment, the liquid medium can be an aqueous medium. In one illustrative embodiment, the bird's nest or the part thereof is generally submerged in water during ultrasound treatment.

[0011] Without being bound by theory, it is believed that the ultrasound process inactivates (disintegrates) all of the contaminants, for example pathogenic living cells, bacteria, viruses and combinations thereof. For example, these contaminants can be eliminated via shearing of the DNA during ultrasound treatment. Any frequency of ultrasound can be used in the present invention that is greater than a frequency of about 20 KHz (which is considered to be the lower limit of ultrasound) and which preserves the structural integrity of the bird's nest or the parts thereof to be treated. For example, the upper limit may be about 1 MHz to about 2 MHz. In illustrative embodiments, a frequency ranges from about 20 KHz to about 1MHz, such as for example, about 20 KHz to about 800 KHz, about 20 KHz to about 500 KHz, about 20 KHz to about 400 KHz, about 20 KHz to about 300 KHz, about 20 KHz to about 200 KHz, about 20 KHz to about 150 KHz, about 40 KHz to about 800 KHz, about 50 KHz to about 800 KHz, about 70 KHz to about 800 KHz, about 100 KHz to about 800 KHz, about 200 KHz to about 800 KHz, or about 350 KHz to about 800 KHz can be used (See the Examples wherein a frequency range of about 20 to about 40 KHz is used).

[0012] Also the treatment time can be chosen such that contaminants are eliminated to a predefined threshold level (for example 90, 95 or 99 or 99.9 or 100 percent elimination). In one illustrative embodiment, the bird's nest or the part thereof may be subjected to ultrasound for a time period of about 1 minute to about 90 minutes. In illustrative embodiments, the time period may range from about 1 minute to about 80 minutes; about 1 minute to about 60 minutes; about 1 minute to about 45 minutes; or about 1 minute to about 30 minutes, about 1 minute to about 25 minutes, about 15 minutes to about 90 minutes, about 25 minutes to about 90 minutes, or about 40 minutes to about 90 minutes.

[0013] In one embodiment, the method of inactivating a contaminant in a bird's nest or a part thereof may further comprise an inactivation pre-treatment. The pre-treatment may include subjecting the bird's nest or the part thereof to UV radiation. The

pre-treatment may also include exposing the bird's nest or the part thereof to ozone. In some embodiments, the pre-treatment may comprise exposing the bird's nest or the part thereof to UV radiation and ozone. The bird's nest or the part thereof may be subjected to UV radiation at a wavelength of about 100 nm to about 400 nm, about 100 nm to about 350 nm, about 100 nm to about 300 nm, about 100 nm to about 250 nm, about 100 nm to about 200 nm, about 150 nm to about 400 nm, about 200 nm to about 400 nm, or about 300 nm to about 400 nm. (See the Examples wherein a wavelength of about 253.7 nm is used). The bird's nest or the part thereof may be exposed to UV radiation for a time period that may be in the range of about 1 to about 15 seconds, such as for example, about 1 second to about 10 seconds, about 1 second to about 5 seconds, about 5 seconds to about 15 seconds or about 10 seconds to about 15 seconds.. (See the Examples wherein the UV exposure time is 2 seconds). To expose the bird nest or the part thereof to UV radiation, for example, ultraviolet germicidal lamps are placed at a distance from the bird's nest or the parts thereof. This ensures that any contaminants are effectively inactivated. Alternatively or additionally, the bird's nest or the part thereof may also be exposed to ozone. The introduction of ozone sterilizes the bird nest or the part thereof and ensures that any toxic substances and contaminants such as viruses and bacteria are eliminated. The person skilled in the art will therefore appreciate that ozone is generated and introduced under pressure into a chamber containing the bird nest or the part thereof to be decontaminated.

[0014] Any desired contamination that is sensitive to ultrasound can be eliminated using the method of the invention. The contaminant may, for example, be a virus, a bacteria, fungi, protozoa, allergen or any combination thereof. The viruses may be selected from the group consisting of influenza viruses including but not limited to H3N1 viruses or H5N1 viruses; hepatitis viruses; SARS coronoaviruses; herpes viruses; varicella zoster viruses; ebola viruses; gastroenteritis viruses such as rotaviruses, noroviruses, adenoviruses, sapoviruses, or astroviruses; and combinations thereof

[0015] In one embodiment, the degree of inactivation of the contaminant in the bird's nest or the part thereof may be determined by comparing the treated bird's nest or the part thereof with a positive control. The positive control may be a bird's nest or a part thereof containing a reference contaminant, which can be treated or untreated. In another

embodiment, the degree of inactivation of the contaminant in the bird's nest or the part thereof may be determined by comparing the treated bird's nest or the part thereof with a negative control. The negative control may be a bird's nest or a part thereof without a reference contaminant, which can be treated or untreated.

[0016] In some embodiments, at least about 99%, about 90%, about 85%, about 70%, about 50% about 40% or about 30%of contaminants in the bird nest or the part thereof is inactivated upon treatment with ultrasound. The presence of the contaminants can be detected by measuring the intensity of the DNA bands via gel electrophoresis, using molecular cloning methods known to persons skilled in the art.

[0017] The reference contaminant used in the positive controls may be an influenza virus. The term "influenza virus" used in the context of the present specification refers to an RNA virus derived from the family *Orthomyxoviridae*. The *Orthomyxoviridae* family typically comprises 5 types of viruses namely Influenza virus A, Influenza virus B, Influenza virus C, Thogotovirus and Isavirus. It is to be understood that the influenza viruses include both human and non-human influenza viruses such as swine, dogs, cows, and birds. Examples of influenza viruses may include different subtypes such as H3N1, H5N1, H3N2 and H1N1.

[0018] The bird's nest or the part thereof may be placed in an air tight container prior to subjecting the bird's nest to ultrasound. In one embodiment, the air tight container may comprise or consist of a clear and flexible material. The air-tight container may, for example, be a sealable plastic bag. The air-tight container containing the bird's nest or the part thereof may be placed in an outer container, prior to subjecting the bird's nest or the part thereof to ultrasound. The outer container may be rigid and may be made from any suitable material such as plastic or metal. In one illustrative embodiment, the outer container may be comprised of a plastic material. The outer container may also include a padding that protects the bird nests from physical impact. Treating the bird nest while being packed in an inner container and an outer container, optionally provided with a padding, provides for the option to transport the treated bird nest to the customer without further packaging steps

[0019] Any selected amount of the bird's nest or the part thereof can be placed in the air-tight container prior to subjecting it to ultrasound. In some embodiments, a weight

range of about 200 g to about 2 kg of bird's nest or the part thereof, such as for example, about 200 g to about 1500 g, about 200 g to about 1000 g, about 200 g to about 800 g, about 200 g to about 600 g, about 300 g to about 1500 g, about 800 g to about 1500 g, or about 1000 g to about 1500 g can be placed in the air-tight container, prior to subjecting it to ultrasound.

EXAMPLES

The following non limiting examples are also illustrative of the process described above.

Example 1

Preliminary cloning

The following PCR primer pair was used to clone the H3N1 virus DNA for identification of virus presence in bird's nests:

1. H3N1F: 5'-AGC AAA AGC AGG GGA TAA TTC T-3'
2. H3N1R: 5'-CAG TAT GTC TCC CGG TTT-3

The H3N1 viral DNA was then cloned into DNA vectors and subsequently grown in cell culture known to persons skilled in the art.

Example 2

Ultrasound treatment

2 kg of bird's nest cusps were used in the following experiment. A batch of bird's nest cusps were contaminated with H3N1 flu viruses for use as a positive control. Ultrasound treatment was performed using an ultrasonic generator (Advanced Ultrasonic Generator - Operating Frequency: 40k Hz +/- 100 Hz; Output Power: 500W +/- 50W; Transducer Elements: 12 and Weight: 13.6 kg) and 2 immersible transducers (Operating Frequency: 40 kHz +/- 100 Hz; Output Power: 500W +/- 50W; Transducer Elements: 12; Dimensions: 152 x 458 x 84H and Weight: 14 Kg).

Example 3

Pre-Treatment with UV

The bird's nest was sterilized by ultraviolet and ozone methods. For the ultraviolet sterilization, 4 germicidal lamps were placed 3 inches away from the samples, to ensure

that 100% of flu viruses was destroyed. The lamps are G6T6L (GML005) germicidal ultraviolet lamp (253.7 nm) with a current of 420 MA, the output is measured at 120 microwatts/cm^2 per 1 meter. The bird's nests were exposed to UV for 2 seconds.

Example 4

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 20 KHz for 15 minutes. The results are shown in Table 1.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
70%	100%	0%	100%
60%	100%	0%	100%
66%	100%	0%	100%

Table 1

Table 1 illustrates the percentage of viral DNA that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

Example 5

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 20 KHz for 25 minutes. The results are shown in Table 2.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
70%	100%	0%	100%
66%	100%	0%	100%
72%	100%	0%	100%

Table 2

Table 2 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen from Tables 1 and 2 that an increased in exposure time does not significantly affect the percentage of killed viruses.

Example 6

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 30 KHz for 15 minutes. The results are shown in Table 3.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
80%	100%	0%	100%
86%	100%	0%	100%
92%	100%	0%	100%

Table 3

Table 3 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen from Tables 1 and 3 that an exposure to ultrasound at a higher frequency (i.e. 30 KHz) increases the percentage of killed viruses.

Example 7

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean

water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 30 KHz for 25 minutes. The results are shown in Table 4.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
88%	100%	0%	100%
88%	100%	0%	100%
86%	100%	0%	100%

Table 4

Table 4 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen from Tables 3 and 4 that an increased in exposure time does not significantly affect the percentage of killed viruses.

Example 8

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 35 KHz for 15 minutes. The results are shown in Table 5.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
92%	100%	0%	100%
95%	100%	0%	100%
92%	100%	0%	100%

Table 5

Table 5 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen from Tables 3 and 5 that an exposure to ultrasound at a higher frequency (i.e. 35 KHz) increases the percentage of killed viruses.

Example 9

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency 35 KHz for 25 minutes. The results are shown in Table 6.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
95%	100%	0%	100%
95%	100%	0%	100%
98%	100%	0%	100%

Table 6

Table 6 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen from Tables 5 and 6 that an increased in exposure time does not significantly affect the percentage of killed viruses.

Example 10

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 100 g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency 40 KHz for 10 minutes. The results are shown in Table 7.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without treatment	Negative Controls without Treatment
100%	100%	0%	100%
100%	100%	0%	100%
99%	100%	0%	100%

Table 7

Table 7 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen in this example that an ultrasound treatment at a frequency of 40 KHz results in 100% killing of the viruses.

Example 11

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 500g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency 40 KHz for 10 minutes. The results are shown in Table 8.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without Treatment	Negative Controls without Treatment
96%	100%	0%	100%
96%	100%	0%	100%
99%	100%	0%	100%

Table 8

Table 8 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

Example 12

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 500g of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 40 KHz for 15 minutes. The results are shown in Table 9.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without Treatment	Negative Controls without Treatment
100%	100%	0%	100%
100%	100%	0%	100%
100%	100%	0%	100%

Table 9

Table 9 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

Example 13

After being vacuum packed in clear plastic bags which were in turn placed in a respective plastic container, 2 kg of bird nest cusps were submerged in 5 inches depth of clean water and exposed to ultrasound using Advanced Ultrasonic Generator and transducers at a frequency of 40 KHz from Generator and Transducers for 15 minutes. The results are shown in Table 10.

Positive Controls with treatment	Negative Controls with Treatment	Positive Controls without Treatment	Negative Controls without Treatment
100%	100%	0%	100%
100%	100%	0%	100%
100%	100%	0%	100%

Table 10

Table 10 depicts the percentage of viruses that is killed upon ultrasound treatment (see positive controls with treatment). The viral DNA is detected by measuring the intensity of the DNA bands on an electrophoresed gel image using PCR cloning methods.

It can be seen from Tables 9 and 10 that a larger amount of bird's nest can be treated by ultrasound resulting in 100% killing of the viruses.

CLAIMS

What is claimed is:

1. A method of inactivating a contaminant in a bird's nest or a part thereof, the method comprising subjecting the bird's nest or the part thereof to ultrasound.

2. The method according to claim 1, further comprising an inactivation pre-treatment.

3. The method according to claim 2, wherein the pre-treatment comprises subjecting the bird's nest or the part thereof to UV radiation and/or exposing the bird's nest or the part thereof to ozone.

4. The method according to any of claims 1 to 3, wherein the bird's nest or the part thereof is placed in a liquid medium for ultrasound treatment.

5. The method according to any of claims 1 to 4, wherein the contaminant is selected from the group consisting of viruses, bacteria, fungi, protozoa, allergens and combinations thereof.

6. The method according to claim 5, wherein the virus is selected from the group consisting of influenza viruses, hepatitis viruses, SARS coronoaviruses, herpes viruses, varicella zoster viruses, ebola viruses, rotaviruses, noroviruses, adenoviruses, sapoviruses, astroviruses; and combinations thereof.

7. The method according to any of claims 1 to 6, wherein the degree of inactivation of the contaminant in the bird's nest or the part thereof is determined by comparing the treated bird's nest or the part thereof with a treated and/or untreated bird's nest or a part thereof containing a reference contaminant.

8. The method according to any of claims 1 to 7, wherein the degree of inactivation of the contaminant in the bird's nest or the part thereof is determined by comparing the treated bird's nest or the part thereof with a treated and/or untreated bird's nest or a part thereof without a reference contaminant.

9. The method according to claim 8 wherein the reference contaminant is influenza virus.

10. The method according to any of claims 1 to 9, wherein the bird's nest or the part thereof is subjected to ultrasound of a frequency of about 20 KHz to about 2 MHz.

11. The method according to any of claims 1 to 10, wherein the bird's nest or the part thereof is subjected to ultrasound for a time period of about 1 minute to about 90 minutes.

12. The method according to claim 3, wherein the bird's nest or the part thereof is subjected to UV radiation at a wavelength of about 100 nm to about 400 nm.

13. The method according to claim 12, wherein the bird's nest or the part thereof is subjected to UV radiation for a time period of about 1 to about 15 seconds.

14. The method according to any of claims 1 to 13, wherein the bird's nest or the part thereof is placed in an air-tight container prior to subjecting it to ultrasound.

15. The method according to claim 14, wherein the air-tight container consists of a flexible material.

16. The method according to claims 14 or 15, wherein the air-tight container is a sealable plastic bag.

17. The method according to any of claims 14 to 16, wherein the air-tight container is placed in an outer container.

18. The method according to claim 17, wherein the outer container comprises at least one padding therein.

19. The method according to any of claims 14 to 18, wherein the bird's nest or the part thereof is placed in the air-tight container after the inactivation pre-treatment.

20. The method according to any of claims 14 to 19, wherein about 200 g to about 2 kg of the bird's nest or the part thereof is placed in the air-tight container.