

SOFTGEL FORMULATION CONTAINING EDIBLE BIRD'S NEST AND METHOD FOR PRODUCING THE SAME

FIELD OF THE INVENTION

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The present invention relates to a softgel or soft gelatin capsule formulation containing edible bird's nest and a method for producing the same. More particularly, the present invention relates to a softgel or soft gelatin capsule formulation containing a highly concentrated solution of edible bird's nest and optionally resveratrol, and a method for producing the same.

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BACKGROUND OF THE INVENTION

Edible bird's nest is a traditional Chinese delicacy known for its health wellness benefits. It is believed that edible bird's nest nourishes *yin* to moisten dryness, tonifies *qi* (meaning energy in Chinese) and reinforces the functions of the spleen and stomach of a human body. Researchers from the University of Hong Kong have found through *in vitro* studies that edible bird's nest can stimulate cell regeneration and enhance cell-mediated immunity (Ng, M.H., *et al.* (1986), "Potentiation of mitogenic response by extracts of the swiftlet's (*Collocalia*) nest", Biochem Int., 13(3), 521-531).

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A chemical analysis of edible bird's nest soup conducted by some researches revealed that there is a water-soluble glycoprotein in the nest which promotes cell division within the immune system (Kong, Y.C., *et al.* (1987), "Evidence that epidermal growth factor is present in swiftlet's (*Collocalia*) nest", Comp Biochem Physiol B, 87(2), 221-226).

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Edible bird's nest contains mainly amino acids, carbohydrates and mineral salts. Research has shown that for every 100g of dry edible bird's nest, it contains about 49.9g of water-soluble protein (including amido nitrogen, monoamine nitrogen, non-amino nitrogen, arginine, humin, histidine, lysine and cysteine), 30.6g of carbohydrate (glycoprotein and mucin), 4.9 g of iron, 2.5 g of inorganic salt (including potassium, sodium, calcium, magnesium, sulfur, phosphorus, silica and other trace

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elements), and 1.4 g of fiber (Dictionary of Traditional Chinese Medicine, The History of Chinese Medicine and the Nutrition Table).

Five types of mannoses were found in edible bird's nest and these include D-mannitose, D-galactose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, and N-acetyl neuraminate. Glycoproteins can activate white blood corpuscle in human body and is able to produce and synchronies some acids for fighting against bacteria and virus (Kong, Y.C., *et al.* (1987), "Evidence that epidermal growth factor is present in swiftlet's (*Collocalia*) nest", *Comp Biochem Physiol B*, 87(2), 221-226).

Edible bird's nest has also been reported to contain N-Acetylneuraminic Acid (NANA) which can brighten skin. It also has a special quality for activating growth in body's cell and fixing the epidermal growth factor of the cell (Pozsgay, H. *et al.* (1987), "4,8-anhydro-N-acetylneuraminic acid. Isolation from edible bird's nest and structure determination", *Eur J Biochem*, 162(2), 445-450; Kong, Y.C., *et al.* (1987), "Evidence that epidermal growth factor is present in swiftlet's (*Collocalia*) nest", *Comp Biochem Physiol B*, 87(2), 221-226).

Edible bird's nest is known for its many health benefits. In the past decade, people have become more health conscious. Given a choice, many consumers would want to consume edible bird's nest as often as they want to for its health benefits. However, consumers may find it cumbersome to consume edible bird's nest while they are away from home as edible bird's nest is usually packed and sold in glass bottles, in liquid form. Such packaging discourages consumers from carrying the edible bird's nest with them when they are away from home or travel overseas as the bottles can be bulky, heavy and breakable.

Another problem associated with consuming this traditional Chinese delicacy is that there are various types of edible bird's nests available in the market. They come from different regions, different sources and in different types and grades. The health benefit from consuming these edible bird's nests varies with the quality of the edible bird's nest. It would be difficult to assure consistency in the health benefit from consuming the edible bird's nest if there is no consistency in the quality of the edible bird's nest consumed.

It is also found that the traditional methods employed in processing edible bird's nest may result in the loss of some nutritional values in the edible bird's nest.

Resveratrol (trans-3,4',5-trihydroxystilbene) is a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi. Studies have shown that resveratrol has cardio-protective and anti-cancer properties. According to some researchers, resveratrol also shows great potential in extending human lifespan. In laboratory experiments, resveratrol has shown to extend the lifespan of simple organisms, such as yeast and fruit flies.

As a natural polyphenolic compound of the stilbene family, resveratrol has been strongly indicated to have protective effects against cardiovascular disease and, in particular, cancer, including all stages of carcinogenesis (Jang, M. *et al.* (1997), "Cancer chemopreventive activity of resveratrol, a natural product derived from grapes", *Science*, 275(5297), 218-220; Bhat, K.P. and Pezzuto, J.M. (2002), "Cancer chemopreventive activity of resveratrol", *Ann N Y Acad Sci*, 957, 210-229) through their interfering action with a variety of cellular mechanisms at low micromolar concentrations such as (1) inhibition of metabolic activation of procarcinogens; (2) induction of carcinogen-detoxifying enzymes; and (3) inhibition of tumor growth by inhibiting inflammatory signals and angiogenesis. Anti-proliferative and cytotoxic effects of resveratrol and other flavonoids were tested in breast cancer (MCF-7), colon cancer (HT-29), and ovarian cancer (A-2780) cells *in vitro*. Resveratrol inhibited the proliferation of MCF-7 and A-2780 cells in a dose-dependent manner. Some flavonoids such as resveratrol can be effective anti-inflammatory agents by inhibition of endogenous prostaglandin synthesis through inhibition of cyclooxygenase (constitutive COX-1 and inducible COX-2) enzymes.

To obtain the positive health effects of resveratrol, typically long-term and/or high levels of consumption are necessary. In today's fast-paced society, consumers are constantly on-the-go. They often have very busy lifestyles and their busy lifestyles demand the convenience of nutritional supplements which are convenient to carry around so that they can consume them on a regular basis.

Consequently, there is a need to provide a formulation and a method for producing the same that seeks to address at least one of the problems described hereinabove, or at least to provide an alternative.

5 SUMMARY OF THE INVENTION

In accordance with a first aspect of the invention, there is provided a softgel formulation comprising polyethylene glycol, polyvinylpyrrolidone and an edible bird's nest extract.

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In accordance with an embodiment of the invention, the formulation further comprises resveratrol.

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In accordance with an embodiment of the invention, the formulation further comprises a plasticizer. In accordance with some embodiments of the invention, the formulation further comprises a surfactant and an emulsifier.

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In accordance with a second aspect of the invention, there is provided a method for producing a formulation containing edible bird's nest extract, suitable for encapsulation in a softgel. The method comprises cleaning raw edible bird's nest using a solution containing a first emulsifier; extracting the edible bird's nest with water in an oven for 24 to 48 hours; subjecting the solution containing the edible bird's nest extract to separation to obtain a supernatant containing the edible bird's nest extract; freeze-drying the supernatant containing the edible bird's nest extract to obtain an edible bird's nest extract in powder form; mixing the edible bird's nest extract in powder form with polyvinylpyrrolidone to form a first mixture; and mixing the first mixture with a second mixture containing polyethylene glycol to obtain a formulation containing the edible bird's nest extract.

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In accordance with an embodiment of the invention, the second mixture further comprises a plasticizer. In accordance with some embodiments of the invention, the method further comprises mixing the first mixture and the second mixture with a third mixture containing a surfactant and a second emulsifier.

In accordance with an embodiment of the invention, the solution containing the first emulsifier contains about 1% emulsifier based on the total weight of solution containing the first emulsifier. In accordance with an embodiment of the invention, the emulsifier contains glycerol polyethylene glycol ricinoleate and fatty acid esters of polyethylene glycol.

In accordance with an embodiment of the invention, the method further comprises freezing the supernatant containing the edible bird's nest extract at a temperature of below -15°C prior to freeze-drying the supernatant containing the edible bird's nest.

In accordance with an embodiment of the invention, the method further comprises subjecting the raw edible bird's nest to chemical profiling to determine the quality of the raw edible bird's nest.

In accordance with an embodiment of the invention, the method further comprises standardizing the edible bird's nest extract using biomarkers. In accordance with an embodiment of the invention, the biomarkers are N-acetylneuraminic acid, mixed standard of galactosamine, glucosamine, galactose and mannose, amino acids and hormones.

Brief Description of the Drawings

The above and other advantages and features of this invention are described in the following detailed description and are shown in the following drawings:

Figure 1 is a chromatogram of the mixed standard solution obtained in Example 6.

Figure 2 is a chromatogram of sialic acid standard obtained in Example 7.

Figure 3 is a chromatogram of the mixed standard solution of galactosamine, glucosamine, galactose and mannose obtained in Example 7.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a softgel or soft gelatin capsule formulation containing edible bird's nest and a method for producing the same. More particularly, the present invention relates to a softgel or soft gelatin capsule formulation containing a highly concentrated solution of edible bird's nest and optionally
5 resveratrol, and a method for producing the same.

The term "edible bird's nest" as used herein refers to nests built by *Collocalia cephala* swiftlets using the saliva of the swiftlets. It includes all types of edible bird's nest that are available commercially. These include, but are not limited to, house bird's nest
10 and cave bird's nest.

"House bird's nest" refers to bird's nest built using the saliva of swiftlets in swift houses which are used by swifts for nesting.

15 "Cave bird's nest" refers to bird's nest built using the saliva of swiftlets in natural stone walls or limestone caves.

In one embodiment of the invention, the softgel or soft gelatin capsule formulation comprises polyethylene glycol, polyvinylpyrrolidone and an edible bird's nest extract.
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In another embodiment of the invention, the softgel or soft gelatin capsule further comprises resveratrol.

Preferably, the polyethylene glycol in the softgel formulation is present in an amount
25 of about 50% to 70% by weight of the total weight of the softgel formulation.

Preferably, the polyvinylpyrrolidone has an average molecular weight of from about 2,000 to about 54,000 and is present in the softgel formulation in an amount of about 5% to 15% by weight of the total weight of the softgel formulation.
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Preferably, the edible bird's nest extract is present in an amount of about 0.1% to 1.9% by weight of the total weight of the softgel formulation.

The softgel formulation of the present invention may further include a surfactant to
35 increase the bioavailability of the edible bird's nest extract and the resveratrol.

Suitable surfactants include, but are not limited to, polyoxyethylene (20) sorbitan monolaurate, ethoxylated polyglycolized glycerides, linoleic acid, palmitic acid, oleoyl polyoxyl-6 glycerides, olive oil, corn oil, soybean oil, cottonseed oil, palm oil and caprylocaproyl polyoxyl-8 glycerides. Preferably, the surfactant is present in an amount of about 1% to 20% by weight of the total weight of the softgel formulation. In a preferred embodiment, the surfactant is polyoxyethylene (20) sorbitan monolaurate.

The softgel formulation of the present invention may further include various ingredients to help stabilize the formulation or help to promote the bioavailability of the edible bird's nest and the resveratrol, or serve as additional nutrients to a consumer's diet. Such ingredients or excipients may include, but not limited to, a plasticizer and/or an emulsifier.

Suitable plasticizers include, but are not limited to, glycerin, propylene glycol and propylene carbonate. Preferably, the plasticizer is present in an amount of about 1% to 10% by weight of the total weight of the softgel formulation. In one embodiment, the plasticizer is glycerin.

Suitable emulsifiers include, but are not limited to, oleic acid, PEG-35 hydrogenated castor oil, macrogol (25)-cetostearyl ether, polyoxyl 15 hydroxystearate, sorbitan monooleate and sorbitan monostearate. Preferably, the emulsifier is present in an amount of about 1% to 20% by weight of the total weight of the softgel formulation. In one embodiment of the invention, the emulsifier is oleic acid.

In another aspect of the present invention, the softgel formulation of the present invention comprises a softgel gelatin capsule and a fill material within the soft gelatin capsule. In one embodiment of the invention, the fill material comprises polyethylene glycol, polyvinylpyrrolidone and an edible bird's nest extract. In another embodiment of the invention, the fill material comprises polyethylene glycol, polyvinylpyrrolidone, an edible bird's nest extract and resveratrol. The softgel gelatin capsule may be made using any method or manufacturing equipment known in the art. The capsule can be made using shell materials for chewable or swallowable purposes chosen from a blend of materials known in the art.

The edible bird's nest extract of the present invention is present in a highly concentrated form. It is prepared by employing specific parameters, such as temperature, pressure and moisture content as described hereinbelow to preserve the maximum nutritional value of the edible bird's nest in the final concentrated form.

5 Using the method of the present invention and sialic acid as a marker, it is found that the sialic acid content in edible bird's nest can be concentrated by up to approximately 60% as compared to raw edible bird's nest.

10 The softgel formulation of the present invention has several advantages. It contains ingredients that are known to have numerous health benefits. It has better oral absorption and enhances the bioavailability of the active ingredient. It is readily dissolved in the gastric juices of the digestive tract. The softgel is easy to swallow, once swallowed, it releases its contents very quickly.

15 In another aspect of the present invention, a method for producing a formulation containing edible bird's nest extract, suitable for encapsulation in a softgel is provided. The method comprises extracting edible bird's nest with water to obtain an edible bird's nest extract in concentrated form. Preferably, expanded edible bird's nest is used in the extraction process.

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In the extraction process, the expanded edible bird's nest is first processed into smaller pieces. This can be done by any suitable methods, such as blending, tearing or grinding the edible bird's nest. One skilled in the art will recognize that other suitable methods may be employed without departing from the scope of the present invention.

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Water is then added to the processed edible bird's nest to form a solution containing the edible bird's nest. The term "water" as used herein is intended to mean any types of water suitable for consumption. In a preferred embodiment, distilled water is used, i.e. water which has been purified via distillation from non-volatile compounds, e.g. dissolved salts, micro-organisms, etc., which otherwise are present in normal water.

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The solution containing the edible bird's nest is then left in an oven for 24 to 48 hours at a temperature in the range of 75°C to 85°C. After the edible bird's nest is fully

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dissolved in the solution, the solution containing the edible bird's nest undergoes a separation process to remove any undissolved edible bird's nest impurities. The supernatant from the separation process (or the liquid extract containing the edible bird's nest) is collected. Preferably, the solution containing the edible bird's nest is
5 separated by centrifugation, although one skilled in the art will recognise that other suitable separation methods can also be used without departing from the scope of the present invention.

The collected supernatant (or the liquid extract) is kept frozen at below -15°C ,
10 preferably at -20°C for a predetermined duration. Preferably, the liquid extract is kept frozen for a period sufficient for the liquid extract to be fully frozen. This is to enhance the freeze-drying process which will be described in greater details below and to prevent bacteria growth. In an embodiment of the present invention, the liquid extract is kept frozen for at least 12 hours.

15 After the liquid extract is sufficiently frozen, the liquid extract undergoes a freeze-drying process. In this process, the frozen liquid extract is freeze-dried in a freeze-dryer at a temperature in the range of -50°C to -55°C . The duration in which the frozen liquid extract is subjected to the freeze-drying process is dependent on the
20 volume of the frozen liquid extract to be freeze-dried. In one embodiment of the invention, the frozen liquid extract is freeze-dried for about 24 hours.

Before the frozen liquid extract is introduced into the freeze-dryer, the freeze-dryer is preferably sufficiently warmed up to ensure that there is consistency in the pressure
25 and temperature within the freeze-dryer during the freeze-drying process. Preferably, the pressure within the freeze-dryer is set at below 0.10 mbar. The edible bird's nest extract obtained after the freeze-drying process is in dry powder form. The bird's nest extract in dry powder form can be stored at a temperature of about -20°C or lower prior to use.

30 In one embodiment of the invention, the bird's nest extract in dry powder form is mixed with polyvinylpyrrolidone and polyethylene glycol to obtain the softgel formulation of the present invention. In another embodiment of the invention, the bird's nest extract in dry powder form is mixed with polyvinylpyrrolidone, polyethylene
35 glycol and resveratrol to obtain the softgel formulation of another embodiment. In

other embodiments of the invention, the method also comprises mixing other ingredients or excipients to the softgel formulation of the present invention. The other ingredients or excipients include, but are not limited to, plasticizers, surfactants and emulsifiers.

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In an embodiment of the invention, the method comprises mixing the bird's nest extract in dry powder form with polyvinylpyrrolidone to form a first mixture. This is followed by mixing the first mixture with a second mixture containing polyethylene glycol. The second mixture may further include a plasticizer. The plasticizer may be
10 mixed with polyethylene glycol before polyethylene glycol is mixed with the first mixture. The first mixture and the second mixture are mixed with constant stirring. In other embodiments of the invention, a third mixture containing a surfactant and emulsifier may be added to the first and the second mixtures. The final mixture is left to mix to obtain a homogeneous mixture. In a preferred embodiment of the invention,
15 the plasticizer is glycerin. In other preferred embodiments of the invention, the surfactant is polyoxyethylene (20) sorbitan monolaurate and the emulsifier is oleic acid.

In an embodiment of the invention, the raw edible bird's nest is cleaned before it
20 undergoes extraction process. The edible bird's nest is cleaned using a solution containing an emulsifier. In a preferred embodiment, the solution containing the emulsifier contains about 1% emulsifier based on the total weight of the solution containing the emulsifier. It is found that cleaning raw edible bird's nest in a solution containing an emulsifier is effective in removing nitrite from the edible bird's nest to a
25 level of less than 15 ppm.

The cleaning process comprises soaking raw edible bird's nest in water for a predetermined duration. The water is discarded and the edible bird's nest is flushed with more water. The edible bird's nest is then soaked in a pre-heated emulsifier for
30 a predetermined duration. After the soaking is completed, the edible bird's nest is flushed with water again. Physical impurities including feathers are removed from the edible bird's nest.

Suitable emulsifiers that can be used in the solution for cleaning the raw edible bird's
35 nest include, but are not limited to, non-ionic emulsifier or solubilizer. The main

component of the emulsifier or solubilizer includes glycerol polyethylene glycol ricinoleate together with fatty acid esters of polyethylene glycol, being the hydrophobic end of the molecule. The hydrophilic end which is smaller in size consists of free polyethylene glycols and ethoxylated glycerol.

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Due to the different types and grades of edible bird's nest that are available in the markets, it is essential to standardize the quality of the edible bird's nest used in producing the softgel formulation of the present invention so that there is consistency in the quality of the softgel formulation produced in accordance with the method of the present invention. Standardization of the edible bird's nest offers the batch-to-batch reproducibility of the softgel formulation with consistent quality.

In accordance with an embodiment of the invention, the method further comprises standardizing and controlling the quality of the edible bird's nest and the edible bird's nest extract. The method comprises determining the nitrite content of the edible bird's nest. This is preferably carried out on every batch of the edible bird's nests that have been cleaned.

Retention samples are collected and stored at a temperature at or lower than -20°C. The samples are then air-dried at room temperature before the samples are processed into fine particles. An amount of edible bird's nest in fine particles form from each sample is weighed and dissolved in water to form a mixture. The mixture is then sonicated before the mixture is centrifuged. Supernatant is collected and filtered through a syringe filter. The filtrate is then injected onto an ion-chromatography system for analysis with a standard solution.

The method of the present invention further comprises standardizing the edible bird's nest extract obtained in accordance with the method of the present invention using biomarkers. This is preferably carried out on every batch of the edible bird's nests extract obtained in accordance with the method of the present invention. This standardization step is aimed to determine the consistency in the quality of the edible bird's nest extract produced by the method of the present invention. A standardized bird's nest extract has a concentration of marker compound that is known to a high degree of accuracy. Because both the amount of the edible bird's nest that is

extracted and the amount of components that is added to the softgel formulation can be varied, it is possible to compensate for natural variability in the edible bird's nest.

5 The standardization process using known biomarkers in the edible bird's nest comprises collecting samples from each batch of edible bird's nest extract produced and storing the samples at -20°C. All the samples are ground to fine powder prior to preparing different samples with the biomarkers. The known biomarkers in the edible bird's nest include N-acetylneuraminic acid (sialic acid), galactosamine, glucosamine, galactose and mannose. Other markers include the 20 essential amino acids and
10 hormones (testosterone, estradiol and progesterone).

HPLC and/or Ion-chromatography Pro-Cation system are used for the detection and quantification of the biomarkers in the edible bird's nest. The method includes preparing the test sample, injecting the test sample in a chromatographic column,
15 running test chromatogram using a mobile phase, scanning, detecting the biomarkers in the extract by matching retention times of the biomarkers in the test chromatogram with that of standard chromatogram and quantifying the biomarkers. The test sample is prepared by subjecting the test sample to hydrolysis in an acid. The hydrolysate obtained from the hydrolysis is then dried in a vacuum to concentrate the solution.
20 The dried residue obtained from the vacuum concentrating process is then re-dissolved in ultra-pure water to obtain a solution. The solution is diluted before it is injected into a chromatographic column for analysis.

The method of the present invention has several advantages. The method provides
25 a concentrated edible bird's nest extract and this is achieved by heating the edible bird's nest in an oven for a period between 24 and 48 hours. The yield obtained in accordance with the present invention is about 60%. The method of the present invention provides an effective way of removing more nitrite from the edible bird's nest, by about 30% more than using the traditional cleaning method. This is
30 achieved by cleaning the raw edible bird's nest in a solution containing an emulsifier. The method also provides consistency in the quality of the softgel formulation produced in accordance with the method of the present invention.

The following examples are provided to further illustrate and describe particular embodiments of the present invention, and are in no way to be construed to limit the invention to the specific procedures, conditions or compositions described therein.

5 **EXAMPLES**

Example 1 (Cleaning method)

About 1% (w/v) of solubiliser/emulsifier was pre-prepared in distilled water. This
10 solubiliser/emulsifier formed a clear cleaning solution in distilled water.

Raw edible bird's nest was first soaked in distilled water for about 30 minutes. The distilled water was then discarded and the edible bird's nest was flushed with fresh distilled water. The edible bird's nest was transferred to a clean beaker filled with the
15 pre-prepared cleaning solution. The edible bird's nest in the solution was sonicated (at a frequency of approximately 40 kHz) for about 30 minutes at a ratio of 20 gram to 1 litre of cleaning solution. After this step, the cleaning solution was then discarded and the edible bird's nest was again flushed with distilled water. Impurities such as
20 feathers were removed to obtain the cleaned edible bird's nest.

Example 2 (Extraction of edible bird's nest)

In the extraction process, the cleaned edible bird's nest was blended, torn and/or ground into small pieces before distilled water was added. The ratio of the edible
25 bird's nest to distilled water was about 1:20 (wt/wt). The edible bird's nest in the solution of distilled water was then heated in an oven at about 80°C for 24 to 48 hours. After the edible bird's nest was fully dissolved in the solution, the extract was collected by centrifugation at 4,000xg for 20 minutes. The collected extract (in
30 solution form) was kept frozen in a freezer for at least 12 hours before the edible bird's nest underwent freeze-drying process.

Example 3 (Freeze-drying of the edible bird's nest)

Before the freeze-drying process began, the freeze-dryer was warmed up for at least
35 20 minutes. The frozen edible bird's nest was added to the freeze-dryer. The frozen

edible bird's nest can either be added to the connector of the freeze-dryer or left inside the chamber of the freeze-dryer. In this example, the frozen edible bird's nest was left inside the chamber of the freeze-dryer. The edible bird's nest was left inside the freeze-dryer for about 24 hours at a temperature of about -50°C to -55°C. The vacuum pressure of the freeze-dryer was set at below 0.10 mbar. A dry powder form of the edible bird's nest was obtained after the freeze-drying process.

Example 4 (Softgel formulation)

The freeze-dried edible bird's nest powder was weighed and mixed with a commercially available solubilizer, polyvinyl pyrrolidone (PVP) (or povidone). The mixture of edible bird's nest powder and polyvinyl pyrrolidone (or povidone) was prepared at a ratio of 1:10, making up Mixture A. A low molecular weight of polyethylene glycol and glycerin in a ratio of 67:2 were mixed at an elevated temperature of 70°C, making up Mixture B. Oleic acid and polyoxyethylene sorbitan monolaurate in a ratio of 10:10 were mixed with constant stirring at an elevated temperature of 70°C, making up Mixture C. Mixture A was then added to Mixture B at constant stirring, followed by adding of Mixture C. This softgel filling was then left to mix at constant stirring for another 30 minutes at 70°C.

Example 5 (Softgel formulation containing resveratrol)

The freeze-dried edible bird's nest powder and resveratrol were weighed and mixed with a commercially available solubilizer, polyvinyl pyrrolidone (PVP) (or povidone). The mixture of edible bird's nest powder, resveratrol and polyvinyl pyrrolidone (or povidone) was prepared at a ratio of 1:5:10, making up Mixture A. A low molecular weight of polyethylene glycol and glycerine in a ratio of 62:2 were mixed at an elevated temperature of 70°C, making up Mixture B. Oleic acid and polyoxyethylene sorbitan monolaurate in a ratio of 10:10 were mixed with constant stirring at an elevated temperature of 70°C, making up Mixture C. Mixture A was then added to Mixture B at constant stirring, followed by adding of Mixture C. This softgel filling was then left to mix at constant stirring for another 30 minutes at 70°C.

Example 6 (Standardization of edible bird's nest)

Before the edible bird's nest underwent extraction process, the cleaned edible bird's nest was analyzed to determine the level of the nitrite content in the edible bird's nest. The analysis began by collecting a retention sample of 0.5% and stored the sample at -20°C in a freezer. The retention sample was air-dried at room
5 temperature prior to the analysis.

(a) Preparation of Test Samples

The air-dried edible bird's nest sample was pounded into fine particles. About 2.5 g
10 ± 0.0005 g of edible bird's nest was weighed and dissolved in 100 of ultrapure water (Type 1 ultrapure water with resistivity of $18.2 \text{ M}\Omega\cdot\text{cm}$ at 25°C). The mixture was then sonicated for about 30 minutes at a sonicating frequency of 40 kHz. After sonication, the sample was centrifuged at $4,000\times g$ for 20 minutes. The supernatant was then collected and filtered through a syringe filter. The filtrate was then injected
15 onto an ion-chromatography system.

(b) Preparation of Reference Standard Solution

An anion standard solution was obtained from commercial source. The anions
20 consisted of fluoride (F), chloride (Cl), nitrite (NO_2), bromide (Br), nitrate (NO_3), phosphate (PO_4) and sulphate (SO_4). The concentration of each anion was at 1,000 ppm. From the anion standard solution, a 0.5 ml of the solution of each anion was pipetted into a 500 ml calibrated glass volumetric flask. Ultrapure water was used to top up the volume to 500 ml. This made a 10 ppm mixed standard solution. Further
25 dilution was conducted to prepare a 5 ppm mixed standard solution. This was done by obtaining a 25 ml of 10 ppm mixed standard solution in a 25 ml volumetric flask. This solution was then poured into a 50 ml volumetric flask before the solution was topped up with ultrapure water. The lowest concentration of the mixed standard
30 solution was 1 ppm. This was obtained by measuring 10 ml of 5 ppm mixed standard solution using a 10 ml volumetric flask and this solution was then poured into a 50 ml volumetric flask before the ultrapure water was filled. The resulting solution was used as reference standard solution.

(c) Preparation of Reagents

Two running eluent were required for the ion-chromatography analysis, namely the anions eluent and suppressor regeneration solution. The anions eluent was made of 3.2 mM sodium bicarbonate and 1 mM of sodium carbonate. To prepare the eluent, 0.3392 g of sodium bicarbonate and 0.084 g of sodium carbonate were weighed and dissolved in 1,000 ml of ultrapure water. The eluent was then sonicated for about 30 minutes at a sonicating frequency of 40 kHz. Eluent was freshly prepared prior to each analysis. The suppressor regeneration solution was 100 mM sulphuric acid. To prepare the suppressor regeneration solution, 5 ml of concentrated sulphuric acid was pipetted into 500 ml of ultrapure water. The solution was then topped up to a volume of 1,000 ml using ultrapure water.

(d) Ion-chromatographic Conditions

A Metrohm 881 Compact Ion-chromatography Pro-Anions system was used in analysing the nitrite content in the edible bird's nest samples. The column used was MetrosepA Supp 5 with a length of 150 mm and 4.0 μm pore size. The detection mode for anions analysis was conductivity detector with a cell constant of 14.8 $\mu\text{S}/\text{cm}$. During the analysis, a 20 μl of standard solution or sample solution was injected onto the system. The pump was operating at a constant flow rate of 0.7 ml/min with the pump pressure ranging from 8.0 to 9.0 MPa. A mixed standard solution chromatogram was obtained and this is as shown in Figure 1.

From the above tests, it was found that the nitrite content in uncleaned raw edible bird's nest was about 78.7 ppm; a cleaned raw edible bird's nest obtained by traditional cleaning method has a nitrite content of about 36.1 ppm; and a cleaned raw edible bird's nest obtained by the method of the present invention has a nitrite content of about 11.1 ppm. This shows that the method of the present invention is effective in removing more nitrite from raw edible bird's nest (by about 30% more than using the traditional cleaning method), thus able to meet most of the standards imposed by various countries.

Example 7 (Determination of the biomarkers in edible bird's nest)

The standardization process using the known biomarkers in the edible bird's nest was conducted on each batch of the processed and extracted edible bird's nest. A

retention sample of 0.5% was collected and stored at -20°C in a fridge. All the samples were ground to fine powder prior to sample preparation.

Preparation of test samples

5 a) N-acetylneuraminic acid (sialic acid)

10 A 0.0500 ± 0.0005 g of sample was weighed and dissolved in 2.0 ml of 0.1N trifluoroacetic acid or 0.06N hydrochloric acid in a Teflon-line screw cap tube. The sample was hydrolyzed for 1 hour at 80°C. Following the hydrolysis, the solution was vacuum concentrated using a vacuum concentrator (rotor rotate speed of 1,200 rpm and cold trap temperature of -55°C). The vacuum concentrating process took approximately 5.5 hours. The dried residue was then re-dissolved in 1,000 ml of ultrapure water.

15 b) Galactosamine, Glucosamine, Galactose and Mannose

20 A 0.0500 ± 0.0005 g of sample was weighed and dissolved in 2.0 ml of 4.0N trifluoroacetic acid or 6N hydrochloric acid in a Teflon-line screw cap tube. The sample was then transferred to an autoclave and the hydrolysis went on for 3 hours at about 121°C. The steps taken after the hydrolysis were similar to those described in paragraph (a) above.

c) Amino acids

25 A 0.0500 ± 0.0005 g of sample was weighed in a hydrolysis tube and 1.0 ml of the hydrolysis solution (consisting of 6N hydrochloric acid containing 0.1% to 1.0% phenol) was added to the sample. Samples were hydrolyzed at about 110°C for 24 hours in a vacuum or inert atmosphere (nitrogen or argon gas) to prevent oxidation. The hydrolyzed samples were dried in a vacuum concentrator and reconstitute in a buffer solution (containing about 2% anhydrous sodium citrate, 1% hydrochloric acid, 0.5% thiodiglycol and 0.1% benzoic acid in water and adjusted to a pH of 2).

30

d) Hormones

0.1000 ± 0.0005 g of sample was weighed and dissolved in 2.0 ml of methanol. The mixture was vortex-mixed for 1 minute before it was centrifuged at 4,000xg for 20 minutes. The supernatant was collected and filtered through a 0.22 µm PTFE syringe filter before being injected into a HPLC system.

5

Preparation of standard solution

a) N-acetylneuraminic acid (sialic acid)

10 A 10 ppm of sialic acid standard was prepared by pipetting 1.25 ml of the sialic acid stock solution (400 ppm) into a 50 ml volumetric flask. The solution was then top-up-to-volume with ultrapure water. The 5 ppm sialic acid standard was prepared by measuring a 25 ml of 10 ppm sialic acid and topped up to 50 ml volume with ultrapure water. A third point standard of 1 ppm was prepared by measuring 10 ml of 5 ppm sialic acid standard and topped up to 50 ml volume
15 with ultrapure water.

b) Mixed standard of Galactosamine, Glucosamine, Galactose and Mannose

20 To prepare a 10 ppm of mixed standard (consisted of galactosamine, glucosamine, galactose and mannose), 4.28 ml of galactosamine (stock concentration of 116.7 ppm), 0.50 ml of glucosamine (stock concentration of 1,000 ppm), 0.50 ml of galactose (stock concentration of 1,000 ppm) and 0.50 ml of mannose (stock solution of 1,000 ppm) were pipetted into a 50 ml volumetric flask. The mixture was then topped up to 50 ml of volume with ultrapure water.

25

A second concentration point of 5 ppm was prepared by measuring 25 ml of the mixed standard (10 ppm) and topped up to volume to 50 ml with ultrapure water.

30 A third concentration point of 1 ppm was prepared by measuring 10 ml of 5 ppm mixed standard and topped up to 50 ml volume with ultrapure water.

c) Amino acids

The amino acids standard was purchased from a commercial source. The amino acids in the standard were 2.5 mmol/L in 0.1N HCl except that of L-cystine which was at 1.25 mmol/L. The amino acids in the standard were L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine and ammonium chloride. The amino acids standard was diluted to the desired concentration of 0.5 mmol/L.

d) Hormones

The standard solution of testosterone was prepared in methanol. Testosterone standard of 0.1500 ± 0.0005 g was weighed and added to a 100 ml of methanol. This was the first stock solution with concentration of 1,500 µg/ml. From 1,500 µg/ml, the solution was further diluted 40 times to a secondary stock solution with concentration of 37.5 µg/ml. The working standard solution was prepared in diluting the secondary stock solution 100 times to obtain the final working standard solution concentration of 375 ng/ml. This working standard solution was prepared in the eluent used in running the analysis and was prepared fresh every time before each analysis.

Progesterone primary standard solution was prepared by weighing 0.2000 ± 0.0005 g of progesterone standard and adding it to a 100 ml methanol. The concentration obtained for this primary standard solution was 2,000 µg/ml. A secondary standard solution was prepared by diluting the primary standard solution 100 times to obtain a concentration of 20 µg/ml. This was further diluted to 500 ng/ml (40 times dilution) with the eluent prepared for progesterone analysis. The working standard solution for progesterone was prepared fresh before each analysis.

The primary standard solution of estradiol was prepared in acetonitrile. 0.2000 ± 0.0005 g of estradiol standard was weighed and dissolved in 100 ml of acetonitrile. This was the primary standard solution of 2000 µg/ml. The secondary stock solution with the concentration of 20 µg/ml was prepared by diluting the primary standard solution 100 times. A further dilution of 100 times using the eluent prepared for estradiol analysis was used to obtain the working

standard solution of 200 ng/ml. The working standard solution for estradiol was prepared fresh prior to analysis.

Preparation of eluent

5 a) N-acetylneuraminic acid (sialic acid)

10 The eluent for chromatography separation for sialic acid was a mixture of 20 mM of sodium hydroxide and 100 mM sodium acetate solution. To prepare the eluent, a 0.8000 ± 0.0005 g of sodium hydroxide pellet and 8.2000 ± 0.0005 g of anhydrous sodium acetate was weighed and dissolved in 1,000 ml of ultrapure water. The eluent was then sonicated at a sonicating frequency of 40 kHz for 30 minutes. The eluent was prepared fresh for each analysis.

15 b) Mixed standard of Galactosamine, Glucosamine, Galactose, Mannose

The eluent for the mixed standard of galactosamine, glucosamine, galactose and mannose consisted of 20 mM of sodium hydroxide and 1 mM of sodium acetate. To prepare the eluent, a 0.8000 ± 0.0005 g of sodium hydroxide pellet and 0.0820 ± 0.0005 g of anhydrous sodium acetate was weighed and dissolved in 20 1000 ml of ultrapure water. The solution was sonicated at a sonicating frequency of 40 kHz for 30 minutes before being used in the analysis. Eluent was prepared fresh for each analysis.

25 c) Amino acids

Eluent of amino acids analysis consisted of 3 main solutions (solution A, B and C). Solution A was prepared by transferring 1.7 g of anhydrous sodium citrate and 1.5 ml of hydrochloric acid to a 100 ml volumetric flask. The mixture was diluted with ultrapure water to volume and mixed well before adjusted to pH 3.0 with 5M hydrochloric acid. Solution B was prepared with transferring 1.7 g of anhydrous sodium citrate and 0.7 ml of hydrochloric acid to a 100 ml volumetric flask. The mixture was mixed and diluted with ultrapure water and adjusted to a pH 4.3 with 5M hydrochloric acid. On the other hand, solution C was a solution with 5% of sodium chloride, 1.9% of anhydrous sodium citrate and 0.1% phenol in ultrapure water and adjusted to a pH of 6.0 with 0.1M hydrochloric acid. 35

d) Hormones

5 The eluent for testosterone consisted of 0.02M sodium dihydrogen phosphate, acetonitrile and methanol at a ratio of 51:47:2 (v/v). Sodium dihydrogen phosphate buffer salt was weighed (2.3996 ± 0.0005 g) and dissolved in distilled water. The eluent was then mixed to the ratio mentioned and adjusted to pH 3.1 with 5M hydrochloric acid.

10 The eluent for progesterone was similar to those eluent for testosterone with only different in the ratio. The ratio of the eluent for progesterone was 31:67:2 (v/v) (0.02M sodium dihydrogen phosphate, acetonitrile and methanol).

Eluent of estradiol consisted of water and acetonitrile at a ratio of 1:1.

15

Chromatographic conditions

a) N-acetylneuraminic acid (sialic acid) and Galactosamine, Glucosamine, Galactose, Mannose

20 A Metrohm 881 Compact Ion-chromatography Pro-Cation system for carbohydrate analysis was used in the analysis of sialic acid, galactosamine, glucosamine, galactose and mannose in the edible bird's nest samples. The column used was Metrosep Carb1 with a length of 150 mm and 4.0 μ m pore size. The analysis ran at a column temperature of 30°C. The detection mode for the

25 carbohydrate analysis was Pulse-Amperometric Detector with amperometric wall-jet cell. The detector cell operated at a temperature of 32°C. During the analysis, a 20 μ l of standard solution or sample solution was injected onto the system. The pump was operating at a constant flow rate of 1.0 ml/min with the pump pressure ranging from 6.0 to 7.0 MPa. The chromatogram of the sialic acid standard

30 obtained is as shown in Figure 2 while the chromatogram of the mixed standard solution of galactosamine, glucosamine, galactose and mannose obtained is as shown in Figure 3.

b) Amino acids

A Metrohm 886 Professional Thermostat/Reactor with the 887 Professional UV/VIS detectors equipped with Professional IC cation – HP gradient and extension IC pump was used for the analysis of amino acids in the edible bird's nest samples. Separation was achieved on Metrosep Amino Acid 1 column with
 5 a column length of 100 mm and pore size of 4.0 μm . Flow rate was set at 0.4 ml/min and the column temperature was set at 60°C. The post-column reaction involved ninhydrin reagent running at a flow rate of 0.2 ml/ml and at a temperature of 120°C. Detection wavelength was set at 570 nm.

10 c) Hormones

The analysis of hormones was conducted using a high performance liquid chromatography (HPLC) system equipped with UV/VIS and fluorescence detector. For testosterone and progesterone analysis, the column utilized was
 15 made of C-18 packing material with a column length of 150 cm x 4.6 mm ID. The column pore size was 4 μm . Detection was achieved at 240 nm (UV detector) and the flow rate was set at 1.0 ml/min.

Estradiol on the other hand was separated using a longer C-18 column (250 cm x
 20 4.6 mm ID, 4 μm). The molecule was detected using the fluorescence detector operating at an excitation wavelength of 280 nm and emission wavelength of 312 nm. Flow rate was set at 1.0 ml/min.

From the tests carried out on the samples, it was found that the content of sialic acid
 25 in raw edible bird's nest was 7.0% while in the concentrated edible bird's nest extract, it was 11.2%. This shows that sialic acid was concentrated by approximately 60% as compared to raw edible bird's nest.

Example 8 (Determination of biomarkers in edible bird's nest)

30 Similar to those described in Example 7. The biomarkers tested in this example include those of the carbohydrate molecules (sialic acid, galactosamine, glucosamine, galactose and mannose), amino acids and hormones as these are the major components in edible bird's nest.

The above is a description of the subject matter the inventor regards as the invention and is believed that others can and will design alternative systems that include this invention based on the above disclosure.

CLAIMS

1. A softgel formulation, comprising:

polyethylene glycol;

polyvinylpyrrolidone; and

an edible bird's nest extract having a nitrite content of less than 15 ppm, wherein the edible bird's nest extract is obtainable by cleaning raw edible bird's nest using a solution containing an emulsifier and extracting the edible bird's nest with water in an oven for 24 to 48 hours.

2. The formulation according to claim 1, further comprising resveratrol.

3. The formulation according to claim 1, wherein the polyethylene glycol is present in an amount of 50% to 70% by weight of the total weight of the softgel formulation.

4. The formulation according to claim 1 or 3, wherein the polyvinylpyrrolidone is present in an amount of 5% to 15% by weight of the total weight of the softgel formulation.

5. The formulation according to claim 3, wherein the polyethylene glycol has an average molecular weight of from 2,000 to 54,000.

6. The formulation according to claim 2, wherein the resveratrol is present in an amount of 4% to 6% by weight of the total weight of the softgel formulation.

7. The formulation according to claim 1 or 2, wherein the edible bird's nest extract is present in an amount of 0.1% to 1.9% by weight of the total weight of the softgel formulation.

8. The formulation according to claim 1 or 2, further comprising a plasticizer.

9. The formulation according to claim 8, wherein the plasticizer is present in an amount of 1% to 10% by weight of the total weight of the softgel formulation.

10. The formulation according to claim 9, wherein the plasticizer is glycerin.

11. The formulation according to claim 8, further comprising a surfactant and an emulsifier.

12. The formulation according to claim 11, wherein the surfactant is present in an amount of 1% to 20% by weight of the total weight of the softgel formulation.

13. The formulation according to claim 11, wherein the emulsifier is present in an amount of 1% to 20% by weight of the total weight of the softgel formulation.

14. The formulation according to claim 12, wherein the surfactant is polyoxyethylene (20) sorbitan monolaurate.

15. The formulation according to claim 13, wherein the emulsifier is oleic acid.

16. A softgel formulation comprising:
a soft gelatin capsule; and
a fill material within the soft gelatin capsule, the fill material comprising the formulation according to claim 1 or 2.

17. A method for producing a formulation containing edible bird's nest extract, suitable for encapsulation in a softgel, the method comprising:

cleaning raw edible bird's nest using a solution containing a first emulsifier;

extracting the edible bird's nest with water in an oven for 24 to 48 hours;

subjecting the solution containing the edible bird's nest extract to separation

to obtain a supernatant containing the edible bird's nest extract;

freeze-drying the supernatant containing the edible bird's nest extract to obtain an edible bird's nest extract in powder form;

mixing the edible bird's nest extract in powder form with polyvinylpyrrolidone to form a first mixture; and

mixing the first mixture with a second mixture comprising polyethylene glycol to obtain a formulation containing the edible bird's nest extract having a nitrite content of less than 15 ppm.

18. The method according to claim 17, further comprising mixing resveratrol with the edible bird's nest extract in powder form with the polyvinylpyrrolidone to form the first mixture.

19. The method according to claim 17, wherein the second mixture further comprises a plasticizer.

5 20. The method according to claim 19, wherein the plasticizer is glycerin.

21. The method according to claim 19, further comprising:
mixing the first mixture and the second mixture with a third mixture comprising a surfactant.

10

22. The method according to claim 21, wherein the third mixture further comprises a second emulsifier.

15

23. The method according to claim 21, wherein the surfactant is polyoxyethylene (20) sorbitan monolaurate.

24. The method according to claim 22, wherein the second emulsifier is oleic acid.

20

25. The method according to claim 17, wherein the solution containing the first emulsifier contains about 1% emulsifier based on the total weight of solution containing the first emulsifier.

25

26. The method according to claim 17, wherein the first emulsifier contains glycerol polyethylene glycol ricinoleate and fatty acid esters of polyethylene glycol.

30

27. The method according to claim 17, further comprising:
freezing the supernatant containing the edible bird's nest extract at a temperature of below -15°C prior to freeze-drying the supernatant containing the edible bird's nest.

28. The method according to claim 17, further comprising:
subjecting the raw edible bird's nest to chemical profiling to determine the quality of the raw edible bird's nest.

29. The method according to claim 28, wherein the chemical profiling comprises the steps of:

subjecting the raw edible bird's nest to hydrolysis in an acid to obtain a hydrolysate;

5 drying the hydrolysate in a vacuum;

dissolving the dried hydrolysate in ultra-pure water to obtain a solution; and

subjecting the solution to dilution before subjecting the solution to chromatographic analysis.

10 30. The method according to claim 17, further comprising:

standardizing the edible bird's nest extract using biomarkers.

31. The method according to claim 30, wherein the biomarkers are N-acetylneuraminic acid, mixed standard of galactosamine, glucosamine, galactose

15 and mannose, amino acids and hormones.

32. The method according to claim 17, further comprising:

encapsulating the formulation containing the edible bird's nest extract in a soft gelatin capsule.