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(54) **Title**: PRODUCTION METHODOLOGY OF HYDROLYZED COLLAGEN USING SWIFTLET NEST AS RAW MATERIAL

(57) **Abstract**: Health benefits manifested by consuming collagen/hydrolyzed collagen and swiftlet nest exhibit resemblance of each other. Logical instinct should have linked the duos. However, due to age-old assumptions and cultural misplacement, this relationship have been undergrounded. Swiftlet nest has been consumed by Chinese community as a premium delicacy and effective medicine as well as beauty enhancer. So much so that it has become the food of status and command very high price. Due to this reason, the culture of "if it ain't broken, dont fix it" permeated. Double steaming, sometimes called double boiling, is the only widely used cooking method till today, under the belief that over heating above 100°C will destroy the nutrion value of swiftlet nest. This invention is a breakthrough from tradition. Swiftlet nest is cooked above 100°C, using steam sterilization method. Amino acids analysis has been carried out on the sample of the swiftlet nest cooked using this invention. It shows the amino acids profile does fit the amino acids profile of collagen and hydrolyzed collagen. Hence, with this invention, swiftlet nest proved to be a source rich in amino acids and can be used as the raw material to produce collagen and hydrolyzed collagen.

TITLE

Production Methodology of Hydrolyzed Collagen Using Swiftlet Nest As Raw Material

BACKGROUND LITERATURE

Collagen

Collagen is composed of a triple helix, which generally consists of two identical chains (α1) and an additional chain that differs slightly in its chemical composition (α2). The amino acid composition of collagen is atypical for proteins, particularly with respect to its high hydroxyproline content. The most common motifs in the amino acid sequence of collagen are glycine-proline-X and glycine-X-hydroxyproline, where X is any amino acid other than glycine, proline or hydroxyproline.

Hydrolyzed Collagen

Hydrolyzed collagen is a form of collagen. It is also called collagen hydrolysate, collagen peptide, gelatine, gelatine hydrolysate and hydrolyzed gelatine. The amino acid content of hydrolyzed collagen is the same as collagen. It is water soluble and contains peptides like amino acids as well as glycine, proline, hydroxyproline, and glutamic acid, which help form new collagen in the body.

In cosmetics, hydrolyzed collagen may be found in topical creams, acting as a product texture conditioner, and moisturizer.

Digestibility. The bioavailability of hydrolyzed collagen was demonstrated in a 1999 study; mice orally administered hydrolyzed collagen digested and absorbed more than 90% within 6 hours, with measurable accumulation in cartilage and skin. A 2005 study found hydrolyzed collagen absorbed as small peptides in the blood.

Skin Health. A preclinical study investigated the effects of oral ingestion of hydrolyzed collagen, along with vitamin C and glucosamine, suggested that the moisture content of skin, its viscoelastic properties, and smoothness benefit. The mechanism of action of ingested hydrolyzed collagen on skin may be the increased density of collagen fibrils and the fibroblasts' density (the fibroblasts being the main cells of the dermis, and those producing collagen). It may be that that the peptides of ingested hydrolyzed collagen have chemotactic properties on fibroblasts or an influence on growth of fibroblasts.

Joint & Bone Health. Some clinical studies report that the oral ingestion of hydrolyzed collagen decreases joint pain, those with the most severe symptoms showing the most benefit. Beneficial

action is likely due to hydrolyzed collagen accumulation in the cartilage and stimulated production of collagen by the chondrocytes, the cells of cartilage. Several studies have shown that a daily intake of hydrolyzed collagen increases bone mass density. It seems that hydrolyzed collagen peptides stimulated differentiation and osteoblasts activity- the cells that build bone - over that of osteoclasts (cells that destroy bone).

However, other clinical trials have yielded mixed results. In 2011, the European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies concluded that "a cause and effect relationship has not been established between the consumption of collagen hydrolysate and maintenance of joints". Four other studies reported benefit with no side effects; however, the studies were not extensive, and all recommended further controlled study. One study found that oral collagen only improved symptoms in a minority of patients and reported nausea as a side effect. Another study reported no improvement in disease activity in patients with rheumatoid arthritis. Another study found that collagen treatment may actually cause an exacerbation of rheumatoid arthritis symptoms.

Efficacy assertions. It has been claimed that hydrolyzed collagen may promote lean muscle mass through the burning of fat rather than carbohydrates and proteins, toning and thickening skin, joint rebuilding, arterial strengthening, increased energy, organ rebuilding, alleviate osteoporosis, as well as lessening the symptoms of arthritis, high blood pressure, bladder weakness, chronic fatigue, shallow breathing, autoimmune, skin problems, and splitting nails.

Swiftlet

Swiftlet are birds contained within the four genera Aerodramus, Hydrochous, Schoutedenapus and Collocalia. They form the Collocaliini tribe within the swift family Apodidae. The group contains around thirty species mostly confined to southern Asia, southPacific islands, and northeastern Australia, all within the tropical and subtropical regions.

Its diet consists of flying insects which are caught on the wing. It often feeds in large flocks with other species of swift and swallow. It breeds in colonies in caves, in a cleft in a cliff or sometimes in a building. The bracket-shaped nest is white and translucent and is made of layers of hardened saliva attached to the rock.

Swiftlet's Nest

Less common to the western world, but swiftlet nest is considered as a great delicacy and effective medicine as well as beauty enhancer within the Chinese community throughout the world. The nest used in bird's nest soup are composed almost entirely of saliva with little or no plant material. The soup is made by soaking and steaming the nests in water. When cooked, swiftlet nest have a gelatinous texture.

Swiftlet nest has been used for centuries whether as a tonic or a health food. Consuming swiftlet nest regularly can give a person exuberant physical and mental strength as well as to restore one's youthfulness. The tonic power of swiftlet nest is believed to improve skin complexion and to slow the aging effect.

In Chinese cuisine, high medicinal and aphrodisiac qualities are ascribed to these nests. Scientific

investigations reveal these nests to be high in protein. Many consumers of bird nest soup report significant improvement in appetite.

In Traditional Chinese Medicine (TCM), swiftlet nest is believed to offer good effect for creating consumptive diseases, curing tuberculosis, dry coughs, suppressing cough and phlegm-dyspnea (difficult breathing), alleviating asthma, hemoptysis (coughing blood), improving the voice, asthenia, stomach ulcer, relieving gastric troubles, and general weakness of bronchial ailments. It is also traditionally used to nourish the kidneys, lungs heart and stomach to aid renal functions, raise libido, strengthen the immune system, promote growth, enhance the immune system, improve concentration, increase energy and metabolism, and regulate circulation.

Although swiftlet nest has been traditionally prescribed to cure certain diseases for many generations, the actual characteristics and properties of swiftlet nest has not been thoroughly studied. Its usage and benefits are mainly based on historical, anecdotal and observational reports.

ENLIGHTENMENT

Health benefits manifested by consuming collagen/hydrolyzed collagen and swiftlet nest exhibit resemblance of each other. Logical instinct should have linked the duos. However, due to age-old assumptions and cultural misplacement, this relationship have been undergrounded. Swiftlet nest has been consumed by Chinese community as a premium delicacy and effective medicine as well as beauty enhancer. So much so that it has become the food of status and command very high price. Due to this reason, the spirit of “if it ain't broken, dont fix it” permeated. Double steaming, sometimes called double boiling, is the only widely used cooking method till today, under the belief that over heating above 100°C will destroy the nutrion value of swiftlet nest.

This invention is a breakthrough from tradition. Swiftlet nest is cooked above 100°C, using steam sterilization method. Amino acids analysis has been carried out on the sample of the swiftlet nest cooked using this invention. It shows the amino acids profile does fit the amino acids profile of collagen and hydrolyzed collagen. Hence, with this invention, swiftlet nest proved to be a source rich in amino acids and can be used as the raw material to produce collagen and hydrolyzed collagen.

PROBLEMS TO SOLVE

Raw Material

hydrolyzed collagen available in the market is produced from collagen found in the bones, skin, and connective tissue of animals such as cattle, fish, horses, pigs, and rabbits. All these are animal parts. Although only the by-products are used, this may raise concern to vegetarians and animal activists.

Production Method

Non Organic. The process of hydrolysis involves breaking down the molecular bonds between individual collagen strands using combinations of *heat, acids, alkalis, or enzymes*. Except if the process only involve heat alone, other methods involve the application of chemical components.

Complexity and time consuming. Hydrolyzed collagen refers to enzymatically or chemically processed collagen derived from marine life, though it can also be taken from bovine, ox, pig skin, and bone. Hydrolyzed collagen derived from bovine bone and cartilage involves having the bone crushed, ground, defatted, soaked in acid to remove the calcium, soaked again to break the collagen bonds and then dehydrated. This process results in small, intact amino acids which have not been damaged. These amino acids are quickly absorbed into the bloodstream, and are used as the building blocks of new collagen.

Another common means of producing hydrolyzed collagen are prolonged boiling in a strong acid (acid-HVP) or strong base or using an enzyme such as the pancreatic protease enzyme to simulate the naturally occurring hydrolytic process. With skin-sourced collagen, hides are put in a lime slurry pit for up to 3 months, loosening collagen bonds; the hides are then washed to remove lime, and the collagen extracted in boiling water. The extracted collagen is evaporator concentrated, desiccated with drum driers, and pulverized.

Safety concerns. Hydrolyzed collagen, like gelatin, is made from animal parts, including skin, bones, and connective tissue. It is possible that consumption of hydrolyzed collagen risks contraction of Transmissible spongiform encephalopathy.

The U.S. Food and Drug Administration (FDA), with support from the TSE (Transmissible spongiform encephalopathy) Advisory Committee, has since 1997 been monitoring the potential risk of transmitting animal diseases, especially bovine spongiform encephalopathy (BSE), commonlt known as mad cow disease. The FDA study concluded: "...steps such as heat, alkaline treatment, and filtration could be effective in reducing the level of contaminating TSE agents; however, scientific evidence is insufficient at this time to demonstrate that these treatments would effectively remove the BSE infectious agent if present in the source material."

Nutrition Value

Hydrolyzed collagen produced from the animal parts sources contains 8 out of 9 essential amino-acids, including glycine and arginine - two amino-acid precursors necessary for the biosynthesis of creatine. Glycine and proline concentration is as much as 20 times higher than other food sources of protein. However, it contains no tryptophan and is deficient in isoleucine, threonine,

and methionine, all are essential amino acids, which means that humans cannot synthesize it, so it must be ingested.

THE SOLUTION

Raw Material

It is commonly perceived swiftlet nest is full of protein. Due to traditional assumption that swiftlet nest should not be cooked at high temperature to avoid nutrition loss, swiftlet nest is therefore cooked using double steaming, also called double boiling, a Chinese cooking technique to prepare delicate food. This could be the reason that up to the present time, protein from swiftlet nest has not been widely studied and researched.

This invention bypasses orthodox assumption, by applying comtemporary cooking method, and discovered swiftlet nest is an alternative (if not better) source of amino acids, hence collagen and hydrolyzed collagen.

Production Method

This invention introduce a production methodology which involves pre-cooking procedures and high temperature cooking procedures to produce hydrolyzed collagen from swiftlet nest.

Organic. The raw material, swiftlet nest is hydrolyzed using heat only. No acids, alkalis, enzymes or any cheminal compound is needed to produce hydrolyzed collagen.

Simple and time efficeint. Swiftlet nest is soaked in water for 8 hours**,** then drain dry. Once the soaking water is fully drained and replaced with solvent base, it is cooked using pressure cooking method. Total cooking time is about 1 hour.

Pressure cooking allows food to be cooked with greater humidity and higher temperatures than possible with conventional boiling or steaming methods. In an ordinary non-pressurised cooking vessel, the boiling point of water is 100 °C (212 °F) at standard pressure; the temperature of food is limited by the boiling point of water because excess heat causes boiling water to vaporize into steam. In a sealed pressure cooker, the boiling point of water increases as the pressure rises, resulting in superheated water. At a pressure of 1 bar or ~15 psi (pounds per square inch) above the existing atmospheric pressure, water in a pressure cooker can reach a temperature of up to 121 °C (250 °F), depending on altitude.

Once cooked and after cooled down in ambient temperature, a hydrolized collagen is ready in vacuum sealed jar. As it is thoroughly sterilised and vaccum sealed, the shelf life could be prolonged without any perservative or special storage requirement, hence ensuring a true organic hydrolised collagen ready for consumption in extended time.

Food safety. One of the major risk of canned/bottled food is botulism poisoining. The poison is produced by Clostridium botulinum, a bacterium that is commonly found in soil, or on raw fruits and vegetables, on meat and fish and many others foods and surfaces. Botulism spores are tough,

and cannot be killed with boiling water or heat without including canning pressures. 116°C is the minimum temperature necessary to destroy botulism spores, and the only way to guarantee safe canning for food items rich in protein such as meats and seafood. Pressure cooking at a holding time of at least 15 minutes at 121 °C (250 °F) at 100 kPa (15 psi), or 3 minutes at 134 °C (273 °F) at 100 kPa (15 psi) will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant.

Since the finished product is readily vacuum sealed in the jar, the contamination risk is reduced to the minimal as no additional steps are needed to transfer and pack the finished product.

With this invention, it accomplishes three important functions of hydrolyzed collagen production in one single process: PROTEIN DENATURATION, STERILIZATION, AND VACUUM SEALING.

Nutrition Value

Latest amino acid analysis using swiftlet nest as raw material and produced with pressure cooking method shows 18 amino acids are present. Refer attached SGS report.

Compared to hydrolyzed collagen available in the market, tryptophan is absence, significant presence of threonine and isoleucine, with similar trace of methionine.

Furthermore, this new type of hydrolised collagen is ready-to-eat, no additional steps are required that may cause possible nutrition loss and contamination.

CLAIMS

1. Swiftlet nest used as raw material for collagen and hydrolyzed collagen production.

2. Pandanus Amaryllifolius or its extracted compound, used as ingredient for the solvent base of collagen and hydrolyzed collagen production.

3. Stevia Rebaudiana or its extracted compound, used as ingredient for the solvent base of collagen and hydrolyzed collagen production.

4. Preparation procedure of Pandanus amaryllifolius solvent base used for collagen and hydrolyzed collagen production.

   (a) water soak fresh Pandanus Amaryllifolius leaf at temperature of 20 °c - 25°c for at least 8 hours;
   (b) boiled raw pandanus amaryllifolius leaf for 3 - 5 minutes;
   (c) further poaching at temperature of 71–85 °C for 30 – 45 minutes.

5. Preparation procedure of Stevia Rebaudiana solvent base used for collagen and hydrolyzed collagen production.

   (a) water soak fresh Stevia Rebaudiana leaf at temperature of 20 °c - 25°c for at least 8 hours;
   (b) boiled raw stevia rebaudiana leaf for 3 - 5 minutes;
   (c) further poaching at temperature of 71–85 °C for 30 – 45 minutes.

6. Hydrolyzed collagen production processes of swiftlet nest with pandanus amaryllifolius solvent base.

   (a) Preparation process:
      i. soak swiftlet nest with clean water for 8 hours at temperature of 20 – 25°c;
      ii. pour away the soaked clean water, drain dry for 2 hours at temperature of 20 – 25°c; add pandanus amaryllifolius solvent base and soak for another 4 hours at temperature of 20 – 25°c;
      iii. double-soaked swiftlet nest with pandanus amaryllifolius solvent base is ready to cook.
   (b) Cooking process:
      i. put the ready to cook double-soaked swiftlet nest with pandanus amaryllifolius solvent base into pressure cooker;
      ii. apply heat to the pressure cooker;
      iii. exhausting the pressure cooker for 30 minutes;
      iv. accumulate the pressure in the pressure cooker so that the temperature in the cooker break above 100°C;
      v. hold the temperature above 100°C for at least 18 minutes;
      vi. remove heat from the pressure cooker; decumulate and empty the pressure in the pressure cooker at temperature of 20 – 25°.
      vii. take out the cooked swiftlet nest with pandanus amaryllifolius solvent base from pressure cooker;
      viii. leave the cooked swiftlet nest with pandanus amaryllifolius solvent base in at ambient temperature for 12 hours, a hydrolyzed collagen is produced and is in ready-to-eat form.

7. Hydrolyzed collagen production processes of swiftlet nest with Stevia Rebaudiana solvent base.

   All processes are the same as Claim item 6, except Stevia Rebaudiana solvent base is used instead of pandanus amaryllifolius solvent base.

8. Hydrolyzed collagen production processes of swiftlet nest with Pandanus Amaryllifolius solvent base and Stevia Rebaudiana solvent base.

   All processes are the same as Claim item 6, except Stevia Rebaudiana solvent base is added and used together with Pandanus Amaryllifolius solvent base.

AMENDED CLAIMS
received by the International Bureau on 25 September 2015 (25.09.2015)

1. Pandanus Amaryllifolius or its extracted compound, used as ingredient for the solvent base of hydrolyzed collagen / hydrolyzed amino acids production.

2. Stevia Rebaudiana or its extracted compound, used as ingredient for the solvent base of hydrolyzed collagen / hydrolyzed amino acids production.

3. Preparation procedure of Pandanus amaryllifolius solvent base used for hydrolyzed collagen / hydrolyzed amino acids production.

   (a) water soak fresh Pandanus Amaryllifolius leaf at temperature of 20 °c - 25°c for at least 8 hours;
   (b) boil raw pandanus amaryllifolius leaf at 100°c for 3 - 5 minutes;
   (c) further boiling at temperature of 71–85 °C for 30 – 45 minutes;
   (d) the material is put to cool dowm completely at room temperature;
   (e) boiled Pandanus Amaryllifolius leaf is removed, only the liquid is used as the solvent base.

4. Preparation procedure of Stevia Rebaudiana solvent base used for hydrolyzed collagen / hydrolyzed amino acids production.

   (a) water soak fresh Stevia Rebaudiana leaf at temperature of 20 °c - 25°c for at least 8 hours;
   (b) boil raw stevia rebaudiana leaf at 100°c for 3 - 5 minutes;
   (c) further boiling at temperature of 71–85 °C for 30 – 45 minutes.
   (d) the material is put to cool dowm completely at room temperature;
   (e) boiled Stevia Rebaudiana leaf is removed, only the liquid is used as the solvent base.

5. Hydrolyzed collagen / hydrolyzed amino acids production processes of swiftlet nest with Pandanus Amaryllifolius solvent base.

   (a) Pre-hydrolysis process:
      i. soak swiftlet nest with clean water for 8 hours at temperature of 20 – 25°c;
      ii. pour away the soaked clean water, drain dry for 2 hours at temperature of 20 – 25°c; add Pandanus Amaryllifolius solvent base and soak for another 4 hours at temperature of 20 – 25°c;

   (b) Hydrolysis process:
      i. total hydrolysis process is 60 minutes, divided into 4 phases of different temperature with specific exposure time;
      ii. phase 1 – constant temperature of 100 °c for exposure time of 20 minutes;
      iii. phase 2 – linear temperature increment from 100 °c to 125 °c in 10 minutes;
      iv. phase 3 – constant temperature of 125 °c for exposure time of 20 minutes;
      v. phase 4 – linear temperature decrement from 125°c to 105°c in 10 minutes;

   (c) Post-hydrolysis post:
      i. the hydrolysed material is in vacuum container;
      ii. total post-hydrolysis process is 10 hours, divided into 2 phases of different temperature with specific exposure time and different lux environment;
      iii. phase 1 – temperature of 20 °c – 25°c for exposure time of 4 hours, under 50 to 80 lux environment;

iv. phase 2 - temperature of 15°c for exposure time of 6 hours, under 40 lux or below environment.

6. Hydrolyzed collagen / hydrolyzed amino acids production processes of swiftlet nest with Stevia Rebaudiana solvent base.

   All processes are the same as Claim item 5, except Stevia Rebaudiana solvent base is used instead of pandanus amaryllifolius solvent base.

7. Hydrolyzed collagen / hydrolyzed amino acids production processes of swiftlet nest with Pandanus Amaryllifolius solvent base and Stevia Rebaudiana solvent base.

   All processes are the same as Claim item 5, except Stevia Rebaudiana solvent base is added and used together with Pandanus Amaryllifolius solvent base.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/062056

A. CLASSIFICATION OF SUBJECT MATTER
INV. C08H1/00 A23J1/00 A23L1/0562 C08L89/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C08H A23J A23L C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOWE C ET AL: "Collocalia mucoid: A substrate for myxovirus neuraminidase", ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, ACADEMIC PRESS, US, vol. 95, no. 3, 1 December 1961 (1961-12-01), pages 512-520, XP024813023, ISSN: 0003-9861, DOI: 10.1016/0003-9861(61)90184-9 [retrieved on 1961-12-01]	1
A	the whole document	2-8

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2015	10/02/2015

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/062056

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2014/062056

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