

Revealing the Secret of Soups' Healing Power: Nanostructures and Their Functions

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ABSTRACT

As a major formula in both food and traditional medicine, soup delivers health benefits besides the stomach filling nutrients. The occurrence of non-enzymatic glycation (Maillard reaction) and formation of nanostructures distinguish soups from their raw materials, providing a new explanation of soups' bio-functions. The bioactivity and chemical nature of the Maillard reaction products and nanostructures in soups are reviewed. The artificially formed nanoparticles, consisting the purified glycated polyphenol oxidase, provided the first direct evidence of encapsulation and target delivery capacity of the soup nanostructures. The high performance liquid chromatography, coupled with multi-angle laser light scattering, is an effective approach of separating and identifying the bioactive nanostructures. It indicates that the functional and toxicological analysis of soups as either food or medicine should include the vital impacts of nanostructures. The nanostructure provide not only a new perspective of studying the functional traditional food, but also an excellent model for discovery of next generation drugs.

Key words: soups, Maillard reaction, nanostructure, encapsulation, toxicity what do the soups do?

WHAT DO THE SOUPS DO?

Soups have been an inevitable part of cuisine in almost every civilisation. With a history dates back to 4,000 B.C., soup is probably as old as cooking. The English term 'restaurant' is derived from the French verb *restaurer* meaning 'to restore', which referred to the restorative soup served in the 16th century in France⁽¹⁾. Furthermore, some soups are not only the nutritious and filling food, but also very important as the remedies for different conditions before the age of modern medicines. Even in this post-genome era, herbal soup is the dominant and favourite formula in the practice of traditional Chinese medicines (TCM).

Soups from the food system have been used as folk remedies both in the western and the eastern cultures. Chicken soup, for instance, is nick-named as 'Jewish penecillin' for its healing power against illness and weakness including fever, asthma⁽²⁾ and inflammation⁽³⁾. In western Africa, the pepper soup 'enemudo oji' is used to ease the respiratory conditions⁽⁴⁾. Some similar counterparts have been documented in the eastern countries. In China, the fish (*Crucian carp*) soup is served as a lactation booster to mothers after their labor. In east Asian countries, the freshwater clam (*Corbicula fluminea Muller*) soup is popularly consumed as a liver protecting agent and has its therapeutic effects confirmed by some pharmacological studies^(5,6). The soup of bitter gourd, a daily vegetable, is well-known for its preventive and therapeutic effects against diabetes⁽⁷⁾.

In traditional Chinese medicines, drinking the herbal soups is still the major form of the medication. The total amount of herbs used to prepare the soups took at least 50% of the annual consumption of herbal medicinal

materials in China. Either being prepared from a single herb or a compound prescription of 4 to 20 kinds of herbs, there is an herbal soup for every condition. The soup of *radix Isatidis* (Ban-Lan-Gen), for instance, protects the epithelial cells from the influenza virus infection and therefore cures the seasonal flu⁽⁸⁾. The roots of *Alisma plantago-aquatica* (Ze-Xie), which target at the kidney meridian (Shen-Jing) and bladder meridian (Pang-Guang-Jing), were used in the compound therapy of Ménière's disease and regulation of lipid metabolism⁽⁹⁾. A classic prescription named 'Ma-Xing-Shi-Gan Tang', composed by four ingredients (ephedra, licorice, *Armeniacae Semen* and gypsum), has been clinically proven as an effective tonic for infantile pneumonia and flu⁽¹⁰⁾. Among TCM practitioners, the herbal soups are generally believed to be more effective than the innate plants or their isolated compositions.

WHAT IS IN THE SOUPS?

Prepared by a boiling process, soups are far less condensed than the flesh of soup ingredients in terms of nutrients. What makes soups different from the raw materials and empowers the healing capacities? During the preparation of soups, the occurrence of chemical reactions, immigration of chemical compositions and the change of their physical status must have contributed to the formation of functional soups. Two questions were addressed in an earlier publication aiming to illuminate the nature and meaning of the unique properties of soups⁽¹¹⁾: 1) why are the TCM herbal soups always black in color; and 2) why do we experience the health benefits only with the soups but not the flesh?

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The answers to these questions may rely on the two major changes occurred during the boiling process of soups: 1) the browning Maillard reaction and its advanced products (MRPs), which can be indicated with the reduction of Maillard reactants in the raw herbal materials and the increase of visible light absorbance during the boiling process (Table 1⁽¹²⁻¹⁵⁾); and 2) the formation of colloidal nanostructures (micelles, liposomes), self-assembling by the single molecules extracted from the flesh of plants and/or animals⁽¹¹⁾.

Giving the soups a dark brownish colour, the MRPs derived from foods (bitter melon, longan) or herbal medicines (*radix Isatidis*) conducted cellular protective activities upon their targeting cell types, by acting as the membrane anchored antioxidants or viral infection inhibitors^(8,11).

Table 1. Influences of boiling process on the content of Maillard reactants in the plants (dry weight%). Data adapted from previous publications⁽¹²⁻¹⁵⁾

	Protein	Alkaline amino acids		Reducing sugar	
		Fresh	Boiled	Fresh	Boiled
<i>M. charantia</i>	4.17	1.81	-	4.68	-
<i>radix Isatidis</i>	13.6	4.41	0.83	20.6	14.6
rhizome of <i>A. orientale</i>	20.4	5.09	0.08	3.53	-
<i>Rehmannia glutinosa L.</i>	6.70	1.28	0.24	5.54	37.1
<i>Arillus longan</i>	2.36	0.71	-	60.1	-

FORMATION OF NANOSTRUCTURES

The self-assembled nanostructures were observed in the milky fish soup, freshwater clam soup and herbal soups, with a particles size distribution in the range from 70 nm to 350 nm. The size of these nanostructures is pH-dependent, which indicates the existence of a natural vector for encapsulation, transportation and release of active small compounds in the soups⁽¹¹⁾.

The formation of nanostructures in soups requires the participants of heat-stable molecules, i.g. proteins, polysaccharides, lipids etc., and at a high concentration. However, this is not exclusive to soups. In cellular plasma, proteins often assemble with lipids and nucleic acids to form some aggregates. This phenomenon has been termed as ‘macromolecular crowding’, correlating to its nature of high concentration and complicity in the compositions^(16,17). The addition of sucrose-polymers (Ficoll PM 70) increased the crowdedness of environment and therefore

facilitated the protein assembly^(18,19). Similarly, the constant immigration of proteins, polysaccharides, lipids and MRPs from plant or animal tissues to aqueous solution, during the boiling process of soups, is hypothesised to facilitate the formation of nanostructures.

The hypothesis is elucidated by the following facts. MRPs derived from the reaction between L-arginine and D-glucose resembled nanostructures in the aqueous solution⁽¹¹⁾, whose size exhibits pH-responding property revealing by dynamic light scattering measurements. The nonenzymatic-glycated polyphenol oxidase purified from rhizome of *Alisma orientale* formed nanoparticles after a boiling process (Figure 1), and improved the solubility of 23-acetyl alisol B by encapsulation, revealed by atomic forced microscopy and high performance liquid chromatography⁽²⁰⁾.

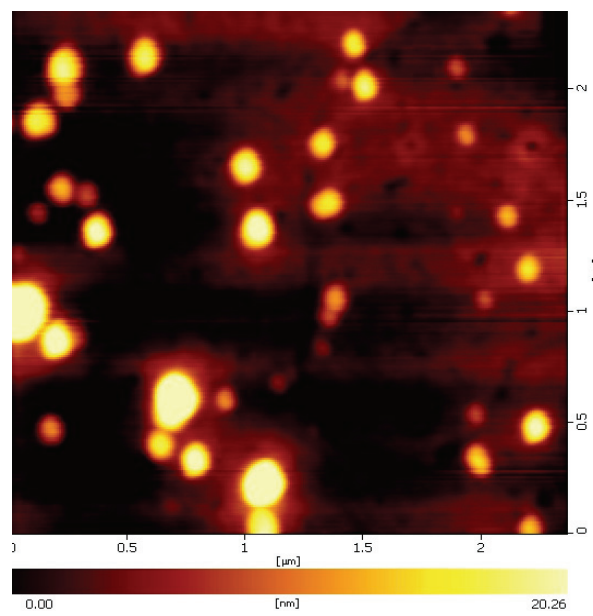


Figure 1. Nanoparticles constructed from glycated polyphenol oxidase and 23-acetyl alisol B examined with atomic forced microscopy (AFM).

ANALYSE THE NANOSTRUCTURES

I. Separation and Identification of Nanoparticles

High performance liquid chromatography (HPLC), often coupled with multiangle laser light scattering (MALLS) monitors, is capable of separating nanoparticles from aqueous suspensions like soups^(21,22). The size, relative molecular weight, shape and surface charge of these separated particles can then be identified and calculated with light scattering methods. As a four-ingredient compound tonic, the Ma-Xing-Shi-Gan decoction is complicated enough as an example of demonstrating the capacity of the HPLC-MALLS approach. The nanoparticles were finely fractionated from the herbal soup with size-exclusion chromatography (SEC, Sephacryl S-1000 column, Amersham Biosciences, USA) with both light scattering and ultra violets

monitors. The fractions containing particles, with the radii of gyration ranged from 50 to 150 nm, were applied to the reversed phase high performance liquid chromatograph (RP-HPLC) for identification of small chemicals. Shown in Table 2, ephedrine and pseudoephedrine from the ephedra, amygdalin from *Armeniaca Semen* and calcium from gypsum have been demonstrated to mainly distribute on the nanostructures, particularly over 99% of ephedrine is encapsulated⁽²³⁾.

The HPLC-MALLS approach has been successfully applied on separation of the nanoparticles from other soup systems, including *radix Isatidis* soup and freshwater clam soup⁽¹¹⁾.

Table 2. The contents of chemicals encapsulated in Ma-Xing-Shi-Gan nanoparticles⁽²³⁾

	Ephedrine ($\mu\text{g/mL}$)	Pseudo- ephedrine ($\mu\text{g/mL}$)	Amygdalin ($\mu\text{g/mL}$)	Calcium cation ($\mu\text{g/mL}$)
Decoction (pH=5)	243.4	109.5	618.7	534.9
Nano- structures	242.6	104.2	541.8	219.6

II. Bioactivities of the Nanoparticles

The nanoparticles separated from clam soup promoted the proliferation of cultured liver cells (L02)⁽¹¹⁾, but acted differently on liver cancer cells (HepG2). The nanoparticles of glycosylated *Alisma orientale* polyphenol oxidase, carrying 23-acetyl alisol B, were labeled with the fluorescent quantum dots and applied on rats by intravenous injection and gavage. Five hours after injection and twenty hours after gavage, the nanoparticles enriched at the sites around both kidneys, while a small portion of particles distributed in the brain of rats received gavage⁽²³⁾. It indicates that the nanostructures are capable of conducting biological activities upon certain type of cells or tissues.

IMPLICATIONS AND FUTURE APPLICATIONS

The two characteristic features of soups, the Maillard reaction and the nanostructures, jointly contribute to the healing power of soups. Apart from their own bioactivities, the nonenzymatic-glycosylated MRPs may play an important, if not dominant, role in the formation of nanoparticles during the boiling process of soups.

As a well-known chemical reaction widely occurred in food and herbal medicine processing, Maillard reaction has been intensively studied for its negative influence on the quality of food and health of human and animal subjects. However, the health benefits behind this reaction and its products have merely been studied systemically.

The bioactive nanostructures in soups demonstrate that it is at least equally as important to know the physical

status of the composing molecules in an aqueous extract, as to prove the existence of these molecules. It is growingly important, in this era of omics when identifying a new molecule has become fairly easy, to know whether the molecules are cooperating as a team rather than act individually.

Apart from boosting the healing power of soups, the nanostructures may have also help to eliminate the toxicity of herbal formulas. Once as the most popular dietary supplement in the States, ephedrine was used to improve physical performance and control body weight. Due to a serial of death-causing side-effect reports, ephedrine was forbidden from selling as dietary supplement. The U.S. FDA consequently issued a maximum dose of 8 mg/kg⁽²⁴⁾. However, according to China Pharmacopeia, the suggested maximum dose of ephedra is equal to more than 90 mg/kg. TCM doctors sometimes instructed even higher dose of ephedra to enhance the efficacy. Surprisingly, few side-effects has been reported in China. As known from Ma-Xing-Shi-Gan soup, over 99% of ephedrine was encapsulated in the nanostructures when ephedra was boiled with other herbs to prepare the medicinal soup. The encapsulation may change the way how ephedrine work, and therefore reduce its toxicity. It indicates the active compounds in soups should be evaluated for their toxicity by testing them in the soup nanostructures rather than in the pure form of these molecules.

This perspective should be instructed to the toxicity studies of acrylamide (ACR) in foods. ACR is derived from Maillard reaction of asparagine and reducing sugars⁽²⁵⁾. In 2002, the baked and roasted foods are found to be rich in neurotoxic and carcinogenic acrylamide, and therefore considered harmful to our health⁽²⁶⁾. Numbers of chemical, toxicological and epidemiological studies were carried out after that, aiming to measure the ACR contents in foods and evaluate the safety of ACR-rich foods⁽²⁷⁻²⁹⁾. To date, instead of a yea-no answer, we find ourselves in a conflict and confusing situation where the epidemiological survey showed no evidence of the high ACR containing food as carcinogenic or toxic, but the toxicological studies in laboratories suggested the opposite^(30,31). Unexpectedly but understandably, this conflict and uncertainty emphasised the safety concerns of ACR, and caused a public panic.

However, a simple fact was overlooked by food scientists and publics: the epidemiologists studied the foods which contains high level of ACR, while the toxicologists were pouring pure ACR into cultured cells or animals. Would it be possible that the conflicts mentioned above are only because we were looking at the different two subjects: the ACR coupled with hundreds if not thousands of other food compositions, and the pure single ACR molecules. ACR, like ephedrine, may acts differently when it is crowded with other Maillard reaction products, macromolecules and bioactive substance in food. It is not too ambitious to anticipate the encapsulation of ACR in the nanostructures formed during the heat processing of food, i.g. coffee, soybean

sauce, etc., will help to eliminate its toxicity.

Nanotechnology has been applied in many aspects of drug discover with either “bottom up” or “top down” strategy. However, the progress is as yet not very satisfying due to the unstable nature of materials, i.e. liposome, or the limit of processing techniques⁽³²⁾. The naturally occurred soup nanostructures provide some excellent models for developing the new drug system, which may have some very attractive properties onboard, i.g. heat-tolerant, improved stability against digestive track, cell or tissue targeting, temperature/pH sensitive and eliminated toxicity. It may be the time for us to rediscover something new from grandma’s kitchen secret recipes.

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