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Degradation of hyaluronic acid derived from tilapia eyeballs by a combinatorial method of microwave, hydrogen peroxide, and ascorbic acid

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ABSTRACT

Hyaluronic acid (HA) derived from tilapia eyeballs was depolymerized by microwave irradiation with ascorbic acid and hydrogen peroxide. Its effects on the structure of hyaluronic acid were determined by ultraviolet spectra (UV) and Fourier transform infrared spectra (FTIR). Molecular weight and the content of glucuronic acid were used as the index for comparison of the different effects of microwave alone, hydrogen peroxide combined with ascorbic acid, and microwave combined with ascorbic acid and hydrogen peroxide. The results showed that HA can be effectively degraded by a combinatorial method of microwave, hydrogen peroxide, and ascorbic acid. The optimal degradation condition was a 1:1 mol ratio between hydrogen peroxide and ascorbic acid at pH 4.0 at 30 min of reaction time and 60 °C of reaction temperature. The low molecular weight oligomeric product of HA was obtained, and the molecular weight of HA was <5 kDa. The content of glucuronic acid in degraded HA was 34.96%, and the yield of the degraded HA was 77.43%. The overall spectral pattern of FTIR and UV did not change between HA and low molecular weight HA (LMW-HA) degraded by the combinatorial method, demonstrating only minor damage to the structure of HA during the degradation process.

1. Introduction

Hyaluronic acid (HA) is a macromolecular polymer consisting of repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine linked by β-(1, 4) and β-(1, 3) glycosidic linkages, respectively [1,2], and the relative molecular weight of hyaluronic acid is in the range from 10^4 to 10^7 kDa [3].

Hyaluronic acid has strong biological activity and is widely used in the food, medicine, and cosmetics industries [4–6]. The biological activities of HA are largely related to its relative molecular weight. It has been reported that low molecular weight hyaluronic acid (LMW-HA) and high-molecular-weight hyaluronic acid have completely different physiological functions, such as regulating the body's immune system [7], inhibiting tumor growth in vivo [8], promoting wound healing [9], etc.

HA extracted from animal tissues and microorganisms usually has a high-molecular-weight. For the range of applications to be expanded, HA must be degraded into LMW compounds. Various methods for the production of LMW-HA have been described, including physical, chemical, and biological [10–13]. Ultrasonic degradation is a well-established procedure that has been applied to HA by several investigators [14]. HA can be depolymerized into LMW-HA and finally into molecules of constant size between 100 and 200 kDa by ultrasonication [15]. By means of the system with hydrogen peroxide (H_2O_2) or hypochlorite and ascorbic acid (Vc), HA can be rapidly degraded into LMW compounds (below 100 kDa), as has been reported in previous studies, when hypochlorous acid, cupric chloride, and ascorbic acid were added to the system, the viscosity of the HA solution dropped rapidly [16,17]. The tilapia eyeball is a potential source of hyaluronic acid, but so far there have been no reports about the degradation of hyaluronic acid derived from tilapia eyeballs. Therefore, the present study was carried out to use the microwave combined with hydrogen peroxide and ascorbic acid to degrade hyaluronic acid derived from

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Table 1
Molecular weight of LWM-HA degraded by each of the four methods.

Methods	Control group	MW 1	MW 2	H_2O_2 + Vc	MW + H_2O_2 + Vc
M_W (kDa)	95.49 ± 0.32[a]	93.76 ± 0.47[b]	93.08 ± 0.16[b]	71.13 ± 1.37[c]	52.67 ± 0.96[d]

Note: Means in the same row with different letter are significantly different ($P < 0.05$), the same case in the following tables.

tilapia eyeballs. The molecular weight and the content of glucuronic acid were used as the index to optimize the conditions of degradation. The effects of the degradation methods on HA structure were observed by UV and FTIR spectra.

2. Materials and methods

2.1. Materials

HA extracted from the eyeballs of tilapia had an average molecular weight (Mw) of 100 kDa. All chemicals were of analytical grade.

2.2. Analytical methods

The relative molecular mass of HA (expressed as Mr) was derived from the kinematic viscosity of the different concentrations of degraded samples in NaCl solution [18,19]. The solution flow time was measured at 25 °C ± 0.2 °C by means of the Ubbelohde (suspended-level) viscometer. Relative viscosity (η_r) was measured according to Equation (1), where t and t_0 were the solution flow time(s) and the solvent flow time(s), respectively, and C is the concentration of natural HA in NaCl solution [g/(100 mL)]. Specific viscosity (η_{sp}) was measured according to Equation (2), followed by linear regression analysis of C_i and η_{sp}/C_i, with the linear intercept being the intrinsic viscosity ([η]). Then, according to Equation (3), the relative molecular mass of hyaluronic acid was measured [19].

$$\eta_r = \frac{t_i}{t_0} \tag{1}$$

$$\eta_{sp} = \eta_r - 1 \tag{2}$$

$$\left[\eta\right] = 3.6 \times 10^{-4} Mr^{0.78} \tag{3}$$

To find the presence of different amino, carboxyl, and hydroxyl groups, we subjected dry powder of HA to infrared spectroscopy. The FTIR spectra were acquired with IRAffinity-1 (Shimadzu, Japan) in the wavelength from 4000 cm^{-1}–400 cm^{-1}. Samples were prepared as a film mixed with one part of the sample and seven parts of dried KBr [3].

HA samples were diluted in distilled water. The concentration of the sample was 0.25 mg/mL. UV–visible absorption spectra of the HA solution were obtained by spectrophotometry at room temperature (UV-1601PC, Shimadzu, Japan) in the range from 190 nm to 400 nm. Distilled water was used as the reference.

Since 100 g hyaluronic acid contained about 46.32 g glucuronic acid, hyaluronic acid content could be determined by measuring the content of glucuronic acid by the Bitter-Muir method [20].

2.3. Degradation of hyaluronic acid

The conditions for microwave irradiation were as follows:

- Condition 1: HA solution was irradiated by a microwave at 60 °C for 20 min, with the solution pH at 4.0.
- Condition 2: HA solution was irradiated by microwave at 100 °C for 20 min, with the solution pH at 4.0.
- Condition 3 (degradation by hydrogen peroxide and ascorbic acid): HA solution was degraded for 20 min by hydrogen peroxide and ascorbic acid, with the H_2O_2:V_C ratio at 2:1, the solution pH at 4.0, and the temperature at 60 °C.
- Condition 4 (degradation by microwave, hydrogen peroxide, and ascorbic acid): HA solution was degraded by microwave at 60 °C for 20 min, with the H_2O_2:V_C ratio at 2:1 and the solution pH at 4.0.

3. Results and discussion

3.1. The effects of different degradation methods

Molecular weight and the content of glucuronic acid were used as the index to compare the effects of microwave irradiation, hydrogen peroxide and ascorbic acid, and microwave irradiation combined with hydrogen peroxide and ascorbic acid on the degradation of hyaluronic acid.

3.1.1. Molecular weight

The different degradation methods had a role in HA degradation (Table 1), but the diversity between and among the different degradation methods was significant. The relative molecular weight of HA was not significantly reduced after only brief microwave irradiation, and the degradation effect was not obvious. When the temperature increased from 60 to 100 °C, the HA molecular weight did not change significantly, and when the degradation temperature reached 100 °C, the HA solution was yellow, therefore affecting the quality of the products. The HA degradations were apparent with the H_2O_2 and Vc method, and with the method of microwave irradiation combined with H_2O_2 and Vc. The relative molecular weights fell rapidly to 71.13 kDa and 52.67 kDa, respectively.

3.1.2. The content of glucuronic acid

HA is composed of glucuronic acid and nitrogen acetylglucosamine. The content of glucuronic acid (GlcA) in HA is constant, so the influence of degradation methods on the structure of HA can be observed by measurement thereof.

The content of glucuronic acid in LMW-HA degraded by the four degradation methods was lower than that in the natural HA, suggesting that the structure of LMW-HA was damaged during degradation (Table 2). The least influence on LMW-HA was exerted

Table 2
The content of glucuronic acid in the LWM-HA degraded by each of the four methods.

Methods	Control group	MW1	MW2	H_2O_2 + Vc	MW + H_2O_2 + Vc
The content of GlcA (%)	40.40 ± 0.19[a]	35.70 ± 0.57[b]	32.12 ± 0.36[c]	34.61 ± 0.04[d]	34.02 ± 0.28[d]