Physicochemical characteristics of rapidly dried onion powder and its anti-atherogenic effect on rats fed high-fat diet

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Running title: Functional characteristics of rapidly dried onion powder

Abstract

Rapidly dried onion (*Allium cepa* L. cv. Momiji No. 3) powder (OP) prepared from the outer layers (from second to fourth scale leaves from the surface) of onion bulbs was analysed for quercetin and polyuronide contents, the effects of enzymatic treatment and the anti-atherogenic effect on rats fed a high-fat diet. Quercetin 4′-glucoside (50%), free quercetin (30%) and quercetin 3,4′-diglucoside (20%) were identified as quercetin derivatives, and boiling-water extraction was effective in extracting these compounds. OP contained 12.9% of polyuronides, the basic skeleton of pectin. Enzymatic degradation (cellulase and pectinase, 50 °C for 12 h, pH 6.0) of OP was effective in obtaining a slurry of smaller particle sizes. Free quercetin increased and the glucosides decreased with enzyme treatment. In Wistar rats fed an OP-added high-fat diet, total cholesterol, HDL-cholesterol and triglyceride concentrations were not significantly different from rats fed a high-fat diet without OP. However, the atherogenic index (AI) of Wistar rats fed an OP-added high-fat diet was lower (AI = 3.3) than rats fed the diet without OP (AI = 4.1). The incremental elastic modulus (IEM) of the aorta from rats fed the OP-added diet was also significantly lower than rats fed the diet without OP. The AI and IEM values of rats fed the OP-added diet were quite similar to the values of rats fed the diet without OP but allowed spontaneous exercise. These results suggest that OP intake is effective for decreasing the risk of arteriosclerosis.

Keywords: Rapidly dried powder, Onion skin, Quercetin derivatives, Polysaccharides, Enzyme treatment, Arteriosclerosis, Rats

1. Introduction

The outer scales of onion (*Allium cepa* L. cv. Momiji No. 3), including the brown skin and outer fleshy scales, are a significant by-product of the industrial preparation of onions. These outer scales are mostly discarded. However, effective utilisation of these is desirable because of the high cost of disposing them. Recently, these scales have been processed into a powdered form produced by high-speed drying apparatus that can pulverise them at a high temperature over a short period. This product has been successfully commercialised in Nagano in Japan. The 'rapidly dried onion powder' (OP) seems to be superior to their intact leaves because it is easier to handle, store and so forth. OP also seems to have a high nutritional value (e.g. protein 6.6%, fat 1.8%, carbohydrates 23.5%, calcium 1.2%, w/w, determined by Japan Food Research Laboratories, Tokyo, Japan) because it contains a large proportion of the edible parts of onion scales and brown skin that have various effective antioxidants (Ly et al., 2005). Therefore, OP is expected to have significant health benefits equalling or surpassing those of onions.

Onion intake has been associated with a reduced risk of coronary heart disease in epidemiological studies, and flavonoids, such as quercetin, are believed to be an important factor affecting this (Knekt, Jarvinen, Reunanen & Maatela, 1996; Knekt et al., 2002). The outer scales of onion contain higher quercetin derivatives (quercetin 4′-glucoside, free quercetin and quercetin 3,4′-diglucoside) than the middle or inner scales, and total quercetin content has been reported to be 10 times higher than that of inner scales (Mogren, Olesson & Gertsson, 2006). Therefore, if OP contains a high amount of quercetin, it may potentially have a high anti-atherogenic function.

In addition to flavonoids, dietary fibre is another important food component that has been associated with

reduced risk of heart disease. Pereira et al. (2004) reported that consumption of dietary fibre from cereals and fruits is inversely associated with risk of coronary heart disease. Pectin, a kind of dietary fibre derived from plant cell walls, has been reported to have a protective effect against atherosclerosis (Baekey, Cerda, Burgin, Robbins, Rice & Baumgartner, 1988). The outer scales of onions may be rich sources of cell wall materials and these components will be concentrated in OP through the drying process. According to the company's data, 5 g of OP can provide 2.8 g of total dietary fibre.

The aim of this study was to assess the utility of OP by analysing physicochemical characteristics and its potential to reduce the risk of coronary heart disease. We investigated (1) quercetin derivatives and polyuronide (pectin) contents of OP, (2) the effect of enzymatic treatment on particle size and quercetin content of OP and (3) the anti-atherogenic effect of OP administered to rats fed a high-fat diet for 18 weeks.

2. Materials and Methods

2.1. Rapidly dried onion powder (OP)

OP was prepared from the outer layer of 'Momiji No. 3' onion bulbs that had been harvested in Hokkaido, Japan, and stored for 6 months. First, surface skins (surface scale leaves) were discarded and from second (coloured) to fourth (fleshy, white) scale leaves from the surface were used for the powder processing using 'Super-high-speed drying apparatus' (Yamaura, Nagano). During the powder processing, the leaves were heated to 200 °C for 15 s and mechanically crushed into fragments of 500–2000 μm, according to the method described by Fukuzawa (2004). A portion of the industrial scale batch (single batch) was used for the

experiments.

2.2 Chemicals

Cyclohexane-1,2-diaminetetraacetic acid (CDTA), guanidine thiocyanate (GTC) and solvents were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Sodium hydrogen carbonate ($NaHCO_3$) was purchased from Wako Pure Chemical Industry, Japan. The acetonitrile and trifluoroacetic acid used were HPLC grade. Water was purified using a Sartorius Arium® 611 (Sartorius Stedim Biotec., Tokyo, Japan).

2.3. Enzymes

Enzymes used were cellulase (E.C.3.2.1.4) (Celluclast® 1.5L) and pectinase (E.C.3.2.1.15) (Viscozyme® L) in liquid form supplied by Novozymes Japan. The enzyme activities of the cellulase and pectinase were checked before use with an Activity Measure Kit of cellulase and rhamnogalacturonan lyase (Megazyme Co.). The cellulase activity was 4300 U/ml, and that of the pectinase was 9700 U/ml (or rhamnogalacturonan lyase).

2.4. Analysis of quercetin derivatives

Extraction efficiency of quercetin derivatives from the OP was compared using different solvents and conditions. Hot water extraction was conducted as follows: OP (1.0 g) and water (30 ml) were mixed in a test

tube, incubated for 30 min in boiling water, sonicated for 10 min then centrifuged (8000*g*, 10 min). The pellet was extracted twice in the same conditions. The three supernatants were combined, filtered, concentrated and then brought to a constant volume (50 ml) with water. Extraction using water or ethanol was conducted in the same manner, except that the boiling treatment was not included. Hot ethanol extraction was also conducted in the same manner except that the sample was extracted in a flask equipped with a reflux condenser. The extraction with each solvent was done triplicate.

For quercetin analysis, aqueous solutions of hot water and water extracts were diluted with an equal volume of ethanol and then filtered through a membrane filter and injected onto HPLC. The HPLC-DAD conditions were as follows: Column, Luna 5 μm C18 (150 × 4.6 mm, Phenomenex Inc., Torrance CA, USA); temperature, 40 °C; mobile phase, (A) 0.1% (v/v) trifluoroacetic acid and (B) 0.1% (v/v) trifluoroacetic acid in acetonitrile; flow rate, 1.0 ml/min. The gradient programme was initiated with 5% (B) and set to obtain 25% (B) at 15 min, 35% (B) at 25 min, 50% (B) at 30 min and 70% (B) at 50 min. Peak detection was performed at 370 nm. An LC-MS system (Quattro Micro API, Waters, Milford, MA, USA) was also used for peak identification.

2.5. Analysis of polysaccharides

A portion of OP (1.5 g) was boiled in 80% (v/v) EtOH (40 ml) for 15 min, cooled with cold water and filtered using Whatman No. 2 filter paper. The residues were sequentially washed with 40 ml each of 80% (v/v) EtOH, 99.5% EtOH and diethylether, then dried under vacuum using an FD-5N freeze-dryer. The resultant powder was weighed and defined as alcohol-insoluble solids (AIS). Triplicate AIS samples were

prepared from OP.

Polysaccharides were sequentially extracted from the AIS using water, chelating agent and alkali solutions (Wang, Pagán & Shi, 2002). The extraction procedure was as follows: AIS samples (100 mg) were soaked and stirred in 20 ml water for 16 h at room temperature, then centrifuged for 10 min at 4 °C and 8000*g*. The residue was washed with 20 ml water, centrifuged again and the supernatants from the two centrifugations were combined. This water-soluble fraction was increased to a volume of 200 ml. The water-insoluble residue was then soaked and stirred in 10 ml of 0.05 M CDTA for 6 h at room temperature, and centrifuged. The resulting residue was washed and centrifuged twice more with 10 ml of the CDTA solution. The CDTA soluble supernatants, which were enriched in ionically bound pectin, were combined then dialysed overnight against distilled water using Visking tubing (MWCO 12,000–14,000 Da). The CDTA-insoluble residue was soaked and stirred in 10 ml of 0.1 M Na_2CO_3 solution at 2 °C for 20 h then at room temperature for 2 h, then centrifuged. The resulting residue was washed and centrifuged twice more with 10 ml of the Na_2CO_3 solution. The Na_2CO_3-soluble supernatants, which were enriched in covalently bound pectin, were combined then dialysed in the same manner as described above. The Na_2CO_3-insoluble residue was soaked and stirred in 10 ml of 6 M GTC solution for 18 h at room temperature, then centrifuged. The resulting residue was washed and centrifuged twice more with 10 ml of the 6 M GTC solution. The 6 M GTC-soluble supernatants were combined and dialysed. The 6 M GTC-insoluble residue was finally soaked and stirred in 10 ml of 4 M KOH solution under N_2 atmosphere for 2 h at room temperature, then centrifuged. The resulting residue was washed and centrifuged twice more with 10 ml of the 4 M KOH solution. The 4 M KOH-soluble supernatants, which were enriched in tightly bound matrix glycans, were combined and dialysed. All dialysates containing the CDTA-, Na_2CO_3-, 6 M GTC- and 4 M KOH-soluble extracts were

made up to a constant volume of 100 ml with water.

The cell wall polysaccharides in each fraction were analysed colorimetrically for polyuronides using the 3,5-dimethylphenol method (Scott, 1979) and for total polysaccharides using the phenol-sulphuric acid method. α-D-Galacturonic acid and D-(+)-glucose were used as standards for the polyuronide assay and the total polysaccharide assay, respectively.

2.6. Enzyme treatment

In a separable flask, 10% and 20% (w/w) of OP was suspended in 100 ml water and the pH was adjusted to 6.0 by adding $NaHCO_3$. The enzyme concentrations tested were 0.5%, 1%, 2%, 4% and 8% (w/v for the substrate). The reaction mixtures were agitated using a mechanical stirrer (220 rpm), while being heated at 50 °C in water bath. The reaction was initiated by adding the enzyme and was stopped by boiling the suspension for 5 min to deactivate the enzyme.

2.7. Fragment analysis

Fragments were investigated using a stereomicroscope (Kenis DAW-TL, Kenis Co.).

2.8. Animal experiment

Eighteen male Wistar rats (9 weeks old) were obtained from CLEA Japan, Inc. (Tokyo) and divided into

three groups (6 rats per group). Group 1 rats were housed individually in cages without an exercise wheel (they could not exercise) and given a high-fat (HF) diet containing 15.1% fat (1.0% cholesterol, 0.5% cholic acid and 10.0% palm oil were added to normal diet (CE-2) that originally contained 4.6% of crude fat). The composition (%, w/w) of other components in the HF were as follows: moisture (7.7), crude protein (22.2), crude fibre (3.3), ash (6.0), Ca (0.9), P (0.9) and nitrogen free extract (NFE; 45.7). Group 2 rats were also given the HF diet but were housed individually in cages with an exercise wheel where they could exercise freely. Group 3 rats were housed individually in cages without an exercise wheel and were given a HF diet containing OP (HF + OP). The composition (%, w/w) of HF + OP diet was as follows: moisture (7.2), crude protein (20.9), crude fat (14.9), crude fibre (3.1), ash (5.6), Ca (0.9), P (0.8), NFE (43.1) and OP (5.0). Groups 1, 2 and 3 were assigned as HF-sedentary group, HF-exercise group and HF + OP–sedentary group, respectively. All rats were kept for 18 weeks in a controlled environment (22 ± 4˚C, 55 ± 10% RH, 12 h light–dark cycle) and allowed free access to water. The rats were sacrificed at the end of feeding period by whole blood collection from the right atrium under anaesthesia with pentobarbital. The blood was collected into heparinised tubes then analysed for plasma cholesterols and triglycerides. After the blood collection, the aortic tissue was prepared according to Nosaka, Tanaka, Asami, Sato and Mitsuo (2003) and analysed for its tensile characteristics. The experiments were approved by the Ethics Committee of Shinshu University.

2.9. Analysis of plasma cholesterols and triglycerides

The plasma was separated from heparinized blood samples immediately after collection and analysed for total cholesterol (total-C), HDL-cholesterol (HDL-C) and triglycerides (TG) using standard enzymatic

colorimetric kits (Total Cholesterol E-Test Wako, HDL-C Test Wako and Triglyceride E-Test Wako) obtained from Wako Pure Chemical Industries (Osaka, Japan).

LDL-C was calculated using the following formula (Noda, Kneyuki, Igarashi, Mori & Packer, 2000):

LDL-cholesterol = total cholesterol – (HDL-C + 0.2 × concentration of triglycerides)

Atherogenic index (AI) was calculated using the following formula (Kayamori and Igarashi, 1994):

AI = (total cholesterol – HDL-C)/HDL-C

2.10. Analysis of arterial stiffness of aorta

A ring-shaped specimen of aorta was stretched in a temperature controlled (24 ± 1 ˚C) saline bath using a Tensile Testing Machine (Toyo Baldwin) as described in the previous paper (Nosaka et al., 2003). The incremental elastic modulus of aorta at an extension ratio of 1.5 was defined as an indicator of arterial stiffness.

2.11. Statistical analysis

The results were expressed as means ± SE. Data from animal experiments were analysed by one-way ANOVA followed by multiple comparison test (Scheffé's test) at the 5% level of significance. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Quercetin derivatives and polysaccharides

Quercetin 4′-glucoside, free quercetin and quercetin 3,4′-diglucoside were the major components of OP in descending order of content (Table 1). The extraction efficiency of these compounds differed according to extraction media and conditions. Ethanol tended to extract free quercetin more than its glucosides; however, hot water extraction was the best method to extract these quercetin derivatives. Therefore, the value obtained by hot water extraction was considered as the true value. When hot water extraction was adopted, quercetin 4′-glucoside, free quercetin and quercetin 3,4′-diglucoside contents were 2.09 mg/g, 1.24 mg/g and 0.83 mg/g, respectively.

Polyuronide and total sugar (total polysaccharides) contents of sub-fractions prepared from AIS are shown in Table 2. The yield of AIS from OP was 51% (w/w). The total polyuronides content of OP was 12.9% (w/w) and the CDTA soluble fraction contained the highest amount of polyuronides (8.0%, w/w). In the total sugar analysis, the KOH soluble fraction contained the highest amount of polysaccharides.

3.2. Enzyme treatment

Considering the cell wall structure, cellulase (Cel) and pectinase (Pec) were applied to degrade the OP. Using 10% and 20% (w/v) dry skin suspension, the enzyme reaction was performed by adding 4% (v/w) enzyme (Cel, Pec and an equal volume mixture, Cel&Pec) for 12 h. This experiment revealed that 10%

substrate concentration degraded the skin to a suitable slurry. At 20% of the dry skin suspension, the reaction mixture was too viscous for mechanical stirring during incubation. Cel, Pec and Cel&Pec enzyme concentrations of 0.5%, 1.0%, 2.0%, 4.0% and 8.0% (v/w) were compared at 10% substrate concentration (12 h). At 0.5%, 1.0% and 2.0 % (v/w) no significant fluid change was observed. Direct observation showed that 4% of each enzyme of Cel&Pec in 10% dry skin suspension was found to give a sufficient degree of slurry formation. The way in which the skins were degraded was observed using a stereomicroscope (Fig. 1). This showed that the enzyme mixture Cel&Pec produced a more transparent picture of pieces than fragments degraded by Pec or Cel. Prolonging the reaction up to 16 or 24 h showed almost no change in morphologies.

Fragment sizes were measured for suspended OP. The measurements were repeated five times and the frequency values are shown in Fig. 2. Distributions of each fragment size were divided into three groups (above 500 μm, 200–500 μm, under 200 μm in width) as shown in Fig. 2. 'Start' refers to the skin suspension at the beginning of reaction. Agitation without enzymes decreased the frequency of fragments above 500 μm from about 60% to 50%, accompanied by an increase in frequency of those under 200 μm from about 10% to 15%, showing the agitation only degraded fragments to some extent. Using Pec, fragments were degraded slightly and with Cel almost no change was observed (Fig. 2). On the other hand, Cel&Pec degraded fragments most prominently, in that the frequency of fragments above 500 μm decreased from about 60% to 30%, while the frequency of fragments less than 200 μm increased from about 10% to 40%. These results are fundamentally in agreement with the previous microscopic observations. The time course of fragment degradation was also investigated (data not shown). Changes in fragment distribution had already begun at 4 h and drastic changes occurred at 12 h, with almost no change being observed at 16 and 24 h. From these results, the reaction time was determined to be 12 h.

Fig. 3 shows the effect of the enzymatic treatment of OP on composition of quercetin derivatives. The enzyme treated sample contained higher quercetin aglycone and lower quercetin glucoside contents than those of the untreated sample. A novel mono-glucoside of quercetin appeared after enzymatic treatment.

3.3. Anti-atherogenic effect

At the end of the feeding period, the body weight of rats fed the HF + OP diet was not significantly different from that of rats fed HF diet (Table 3). However, the body weight of HF-exercise rats was significantly lower than that of the HF group. In the plasma cholesterol analysis, total-C and HDL-C of rats fed the HF + OP diet tended to be lower and higher than rats HF diet, respectively. Plasma TG of the HF + OP group tended to be higher than that of HF group. The AI of the HF + OP group (AI = 3.3) was lower than that of the HF group (AI = 4.1). Moreover, the incremental elastic modulus of the HF + OP group was significantly lower than that of the HF group ($P < 0.05$). In the HF-exercise group rats, plasma TG was significantly lower than that of other two groups and the values of AI and incremental elastic modulus were similar to those of the HF + OP group.

4. Discussion

As predicted, OP was a good resource that contained high amounts of quercetin derivatives and pectin. According to Mogren et al. (2006), the quercetin and its derivatives content (total quercetin content) of onion varied with cultivar, pre- and post-harvest condition and so forth, ranging from approximately 175 mg/kg to

564 mg/kg of fresh weight. In Japan, yellow onions from Hokkaido seem to contain relatively high amount of total quercetin and the average content was reported to be 680 mg/kg (min. 530 – max. 900 mg/kg) (Okamoto et al., 2006). In the current study, the total quercetin content of OP was 4160 mg/kg, and this may be because the quercetin in the outer scales did not degrade significantly during high-speed drying and was concentrated in the powder. It has been reported that quercetin glycosides were degraded by thermal treatment (roasting at 180 °C for up to 60 min) and aglycones were formed (Rohn, Buchner, Driemel, Rauser, & Kroh, 2007). Although OP was subjected to heating at 200 °C during processing, duration of this thermal treatment was only 15 s and most glycoside seemed to be retained. The quercetin derivatives detected in OP were quercetin 4′-glucoside (50%), free quercetin (30%) and quercetin 3,4′-diglucoside (20%). The main compositional difference of quercetin derivatives in OP from that of whole onion was the presence of a large amount of free quercetin in OP. This may reflect a characteristic of the outer scales (Mogren et al., 2006) rather than thermohydrolysis of the glycosides. Polysaccharide analysis revealed that OP contained 12.9% (w/w) of polyuronides, mainly in the form of CDTA-soluble pectin. This suggests that OP is a good source of pectin, comparable to citrus peel, which has been reported to range from 3.6% to 8.6% dry weight (Wang, Chuang & Hsu, 2008).

Enzyme treatment resulted in the following effects. The combined application of cellulase and pectinase was found to be effective in enzymatic degradation of OP (Figs. 1 and 2) to give slurry in which the distribution of fragments less than 200 μm was about 40%. Plant cell walls, including those of onion skin, contain cellulose microfibril interlocked by pectin (polygalacturonic acid and rhamnogalacturonan) and other polysaccharides (Carpita & Gibeaut, 1993). Considering the chemical structure of cell wall, simultaneous cleaving of cellulose microfibrils and pectin seem to cause efficient disruption of the wall. The enzyme

treatment promoted the release of quercetin (aglycone) from their glucosides. A quercetin mono-glucoside that was found in the enzyme-treated specimen was probably the result of the enzymatic hydrolysis of quercetin 3,4′-diglucoside. Generally, quercetin aglycone is considered to be more bioavailable than its glucoside in humans (Wiczkowski et al., 2008).

In the animal experiment, administration of OP together with a HF diet tended to decrease the AI of rats over 18 weeks compared to the administration of HF only. Moreover, the incremental elastic modulus of rats was significantly lowered in HF + OP group than that of HF group. This tendency was similar in that of HF-exercise group. These results indicated that OP could reduce the risk of atherosclerosis caused by high fat intake although exercise seemed to be more important to the risk reduction. In case of HF + OP group, the large amounts of quercetin derivatives and pectin in OP might be associated with the anti-atherogenic effect. In human epidemiological studies, it has been shown that flavonoid intake (such as quercetin) and dietary fibre intake were both associated with reduced risk of atherosclerosis (Knekt et al., 1996, 2002; Liu et al., 2002, Pereira et al., 2004; Estruch et al., 2009). People with higher quercetin intake had lower mortality from ischemic heart disease (Knekt et al., 2002). Ioku, Okuda, Higuchi, Kogirima, & Takei (2008) reported that daily flavonoid intake of Japanese middle-aged women in Kansai area was estimated as 47.4 ± 31.8 mg/d. According to Williamson & Manach (2005), quercetin has a relatively long plasma half-life of 11–28 h, and a 50 mg dose would lead to concentrations of up to ~0.75–1.5 μmol/L in plasma. This plasma concentration may be effective for vascular protection because significant vasodilator responses were observed in the mesenteric bed at concentration of flavonoids (quercetin and its metabolites) of 0.3–3.0 μmol/L (Pérez-Vizcaíno et al., 2002). Concerning this, 12.0 g of OP can provide 50 mg of total quercetin and may increase plasma concentration of flavonoids up to the meaningful level. Moreover, Estruch et al. (2009)

reported that increasing dietary fibre intake with natural foods is associated with reductions in cardiovascular risk factors in a high-risk cohort, and the highest increments in HDL-cholesterol were observed in participants in the upper 20% of fibre intake. Additionally, flavonoids together with pectin might make an important contribution to the anti-atherogenic effect. In the case of apple polyphenol and pectin, Aprikian et al. (2003) reported that pectin and polyphenolic fractions lowered plasma, liver cholesterol and triglycerides in rats, and were more effective together than were either pectin alone or phenolics alone.

In conclusion, rapidly dried onion powder, with high quercetin and pectin contents, has the potential to reduce the risk of coronary heart disease, and this was partly confirmed by the animal experiment. Moreover, enzyme treatment decreased particle size and increased free quercetin, suggesting that the treatment will improve the bioaccessibility and bioavailability of functional components of the rapidly dried onion powder.

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Table 1. Effect of extraction media on the estimation of the contents of quercetin and its derivatives in rapidly dried onion powder

	quercetin	quercetin 3,4′-diG	quercetin 4′-G	Total
Hot water	1.24 ± 0.06	0.83 ± 0.02	2.09 ± 0.06	4.16 ± 0.14
Water	0.28 ± 0.02	0.72 ± 0.01	1.56 ± 0.04	2.56 ± 0.07
Hot ethanol	1.11 ± 0.03	0.41 ± 0.03	1.75 ± 0.04	3.26 ± 0.09
Ethanol	0.85 ± 0.03	0.29 ± 0.02	1.29 ± 0.08	2.43 ± 0.13

Data are expressed in mean value (mg/g powder) ± SE (n = 3).
Abbreviations: diG, diglucoside; G, glucoside

Table 2. Polyuronides and total sugar contents of each fraction extracted from alcohol-insoluble solids of the rapidly dried onion powder

	WS	CDTA	Na_2CO_3	GTC	KOH	Total
Polyuronides	2.9 ± 0.38	8.0 ± 0.27	2.0 ± 0.05	NT	NT	12.9 ± 0.7
Total sugars	4.2 ± 0.16	4.3 ± 0.24	2.1 ± 0.50	1.3 ± 0.19	9.4 ± 0.77	21.3 ± 1.9

Data are expressed in mean value (%, w/w) ± SE (n = 3).

Abbreviations: WS, water soluble fraction; CDTA, 0.05 M cyclohexane-1,2-diaminetetraacetic acid soluble fraction; Na_2CO_3, 0.05 M sodium carbonate soluble fraction; GTC, 6 M guanidine thiocyanate-soluble fraction; KOH, 4 M KOH soluble fraction; NT, not tested.

Table 3. Body weight, total cholesterol, HDL-cholesterol, triglycerides, atherogenic index and incremental elastic modulus of aorta in rats after 18 weeks of a high-fat diet and onion powder-added high-fat diet

Diet	Physical activity	Body weight (g)	Total-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)	AI	IEM (g/mm^2)
HF	Sedentary	537 ± 9.1a	2.62 ± 0.11a	0.53 ± 0.04a	0.81 ± 0.07a	4.1 ± 0.4a	20.0 ± 0.36a
HF	Exercise	484 ± 13b	2.79 ± 0.27a	0.62 ± 0.04a	0.46 ± 0.02b	3.6 ± 0.4a	16.8 ± 0.62b
HF+OP	Sedentary	515 ± 8.5a	2.59 ± 0.11a	0.62 ± 0.06a	0.92 ± 0.10a	3.3 ± 0.4a	17.2 ± 0.42b

Data are expressed in mean value ±SE (n = 6).
Values with different letters are significantly different at $P < 0.05$ (Schefé's test).
Abbreviations: C, cholesterol; TG, triglycerides; AI, atherogenic index; IEM, incremental elastic modulus.

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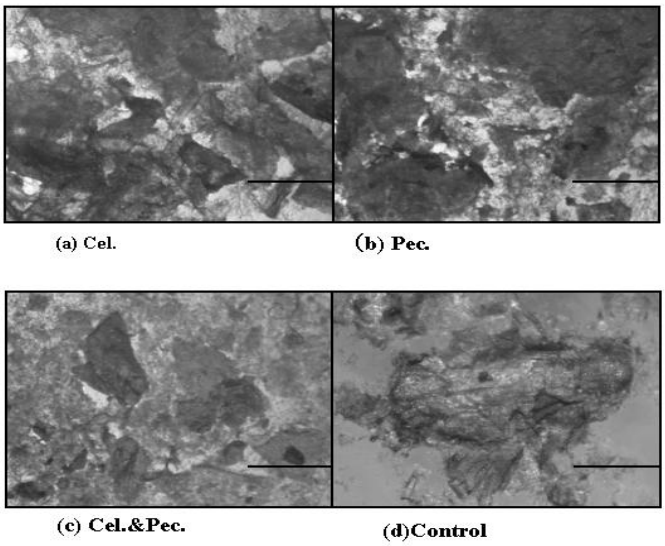

Fig.1. Microscopic observation of skin degradation by enzyme (12 h).

(a), (b) skins degraded only partially by a single enzyme, (c) thick skins degraded to thin semitrasparent debris by two enzymes, (d) control contains thick skins as shown. Control refers to no addition of enzyme (12 h). Reaction conditions were at 10% (w/v) substrate concentration with 4% (v/w to the substrate) of each enzyme. Scale Bar = 500 μm. Cel, cellulase; Pec, pectinase.

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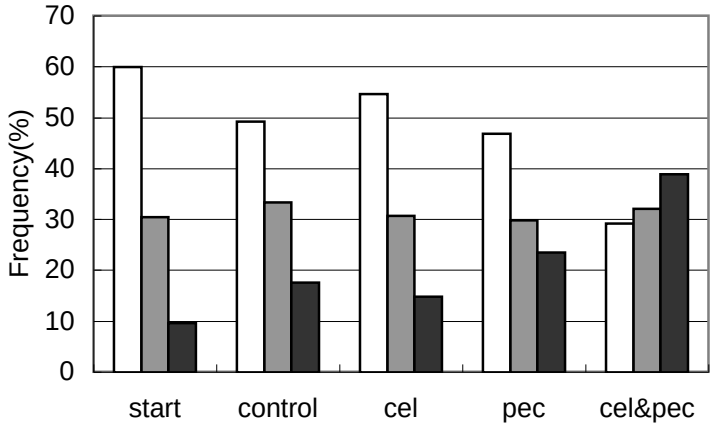

Fig.2. Effect of varied enzymes on degradation of rapidly dried onion powder. The powder was incubated with enzyme for 12 h. 'Start' refers to the skin suspension at the beginning of reaction.

□, ≥500 μm; ▨, 200–500 μm; ■, ≤200 μm

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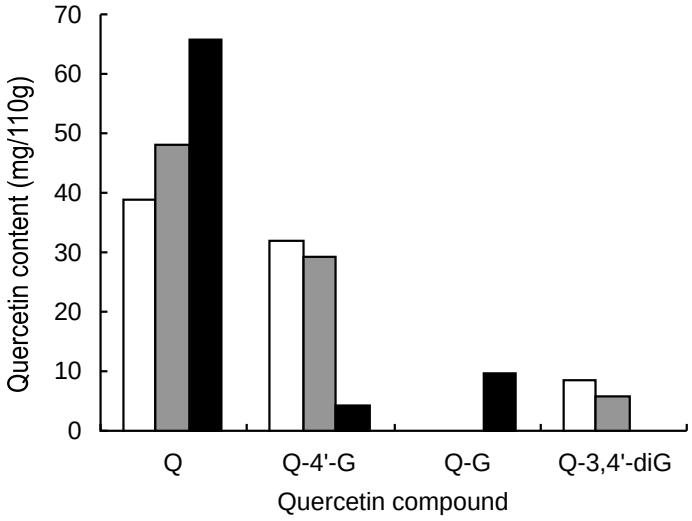

Fig. 3. Composition of quercetin compound of rapidly dried onion powder before (□) and after (■) enzymatic treatment. '□' indicates heating control (incubated without enzyme). Q: quercetin, G: glucoside. Data are means of duplicate analysis.