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Grain-Size Effect on the Structure and Antiobesity Activity of Konjac Flour

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The effect of high-frequency oscillatory type ball-mill treatment on the structure and antiobesity activity of konjac flour was investigated. The grain size of konjac flour changed from 657.3 μm (d_{50}) to 23.7 μm (d_{50}) after 4 h of treatment. The structural change of the konjac flour with different grain size was characterized by using X-ray diffraction (XRD) differential scanning calorimetry (DSC). The results indicated that the crystallinity decreased and the diffraction peak drifted not only by when the crystallization region was reduced but also when the crystalline structure was changed. With the decrease of the grain size and crystallinity, the konjac flour grain, especially the 4 h milled konjac flour, swelled more rapidly and led to the improvement of the antiobesity effect. Compared with the native konjac flour, the 4 h milled konjac flour could significantly decrease the body weight and total wet weight of fat of nutritional obese rats ($P < 0.05$) and also decreased the contents of triglyceride, glucose, and high-density lipoprotein in blood of nutritional obese rat significantly ($P < 0.05$), which meant the grain-size effect of konjac flour improved its antiobesity activity notably.

KEYWORDS: Konjac flour; grain-size effect; structure; antiobesity activity; pulverized model

INTRODUCTION

Konjac flour, a powder from the tuber of the *Amorphophallus* plant, is mainly produced in Southeast Asia and India. After harvesting, the mature konjac tubers, which contain 49−60% konjac glucomannan (KGM), 10−30% starch, 2−5% fiber, 5−14% crude protein, 3−5% soluble sugars, 3.4−5.3% ash, and small amounts of alkaloid, saponin, and stimulating substances in their stem base, were washed, sliced, dried, and milled with a Raymond mill. After that, the mixed power was separated to remove the konjac "dancing powder", so the konjac flour could be obtained (*1*). It contained significant amounts of KGM ranging from 51.3 to 96.9% and small amounts of starch, fiber, and protein (*2*). KGM, a primary polysaccharide component of konjac flour, was a kind of multifunctional natural polymer consisting of β-D-mannose and β-D-glucose residues (molar ratio 1.6:1) linked by β-1,4-glycosidic bonds (*3*, *4*) and has been used as a blend film for different purposes (*5*), interpenetrating polymer networks (*6*), release gel (*7*), drug delivery nanoparticles (*8*), and composite plastics (*9*).

Amorphophallus konjac has also been used in traditional Chinese drugs as an immunoregulation and health-care food for a long time. Its ability to cure some diseases has been well documented (*10*, *11*). First of all, it can delay stomach emptying when taken as an edible *Amorphophallus* konjac-based food, which might lead to a more gradual absorption of dietary sugar, which could reduce the elevation of blood glucose levels, especially after a meal. According to preliminary (*10*) and controlled trials (*11*, *12*), it has been found that blood glucose levels after a meal were lower in patients with diabetes given konjac flour in their food. Second, KGM is a kind of soluble dietary fiber and is essential for human health; like other soluble fibers, it could bind to bile acids in the gut and carry them out of the body in the feces, which requires the body to convert more cholesterol into bile acids (*13*). Finally, it may help weight loss by filling the stomach and making a person feel full. Vuksan et al. (*13*) reported weight loss averaging 5.5 lb in adults when 1 g of glucomannan was taken with a cup of water 1 h before each meal for 8 weeks based on a double-blind study.

However, unlike Vuksans' result, the antiobesity effect of konjac flour was unremarkable due to its large grain size in many studies (*14*). The influence of the grain-size effect on the structure and biological activity was unresolved. In this paper, we treated the native konjac flour with a novel kind of high-frequency oscillatory type mill combined with a temperature control system. The grain-size effect on the structure and antiobesity activity of konjac flour was investigated.

MATERIALS AND METHODS

Materials. Konjac flour was extracted and purified from the tuber of *Amorphophallus konjac* and supplied by Enshi Chuye Konjac Institute, Hubei, China. Its general chemical composition contained 76.6% KGM, 2.1% fiber, 8.3% starch, 1.4% crude protein, 0.8% soluble sugars, and 2.1% ash, which meant that total dietary fiber and soluble and insoluble dietary fibers were 78.7, 76.6, and 2.1%, respectively. Other chemicals, all of reagent grade, were used without purification.

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Ball-Mill Treatment Condition. The ball-mill (oscillatory-type, Wuhan Maike micrometer, Chinese Medicine Rechnology Co., Ltd.; diameter = 13 cm, height = 15 cm) was used; the ball (ceramic, diameter = 1.5 cm) (1.2 kg) and konjac flour (100 g) were placed in the mill. Operation was done at 1500 rpm and 4 °C. The operation time was 0, 1.5, 3, or 4 h, and the samples thusly obtained were coded KF-0, KF-1.5, KF-3, and KF-4, respectively.

Characterization of Ball-Milled and Native Konjac Flour. The granularity was determined by the BT-9300 laser particle size distribution instrument (Dandong, China), the disperse medium adopted absolute ethyl alcohol, an incident angle of 90°, RIF 1.332, λ_{630nm}, and a temperature of 20 °C. The X-ray diffraction (XRD) patterns of the samples were recorded with a Rigaku (Japan) D/max-IIB X-ray diffractrometer and used a CuKa target at 30 kV and 50 mA. The diffraction angle ranged from 50 to 3°. The crystallinity was calculated by the equation (*11*)

$$X_c = F_c/(F_c + F_a) \times 100\%$$

where X_c is crystallinity and F_c and F_a are the areas of crystal and no crystalline regions, respectively.

The differential scanning calorimetry (DSC) analysis of the samples with the weight of ~20 mg was performed by a DSC-822e/400 (Mettler-Toledo) under a nitrogen atmosphere with a flow capacity of 25 mL·min^{-1} from 0 to 400 °C at a heating rate of 10 °C·min^{-1}. The swelling velocity was recorded by the NDJ-8S rotational viscosimeter (Shanhai, China).

Antiobesity Character. *Experimental Animals.* The samples were evaluated with 72 post-weaned 6-week-old Sprague−Dawley rats of ~52.7 ± 2.5 g initial live weight obtained from the Center of Experiment Animal, Tongji Medical University, Hubei, China. Half were male and half, female.

Experimental Animal Feed. The basal feed was composed of 20% barley flour, 10% dehydrated vegetable, 20% soybean flour, 1% yeast, 5% bone powder, 16% corn flour, 10% fish flour, and 2% salt 2%, and the concentrations of protein, carbohydrates, fat, dietary fiber, and energy were 15.2%, 39.7%, 4.9%, 5.2%,and 2.64 kcal·g^{-1}, respectively. The nutritious feed was prepared as in the following proportions: basal feed, 100 g; milk powder, 10 g; meat fat, 10 g; egg flour, 50 g; vitamin A, 1700 units; vitamin D, 1700 units; and soybean sprouts, 25 g; the concentrations of protein, carbohydrates, fat, dietary fiber, and energy were 22.9%, 29.8%, 20.7%, 3.1%, and 3.96 kcal·g^{-1}, respectively.

Procedures. The Sprague−Dawley rats were divided into six groups stochastically on the basis of body weight and sex; the complete plastic metabolism cage was used in the experiment, the temperature and humidity in the air conditioned animal experiment house were 23 ± 1 °C and 45 ± 5%, respectively, with 12 h day and night by turns. The rats could drink the 2.39 mmol·L^{-1} NaCl solution unrestrictedly. The dosage and the feed design are shown in **Table 4**; the weight of the feed was 12 g·day^{-1} in the first 2 weeks and increased 2 g·day^{-1} every week in the residual feed scheduled time (45 days). Such a feed design was to ensure that all of the rats could ingest almost the same amount of feed and were not overdosed; otherwise, the rats might be wan, and the obesity model could not be obtained because of excessive energy intake. The average weight of the obesity model group at the end of experiment was 121.1 ± 22.5 g, twice that of the control group, which is to say the obesity model was successful. The percentage of kilocalories originated from fat included in the experimental diets was 46.9%.

Biochemical Indices. Three milliliters of blood was obtained by cutting the tail, and two drops of blood was used to determine the blood glucose value; the rest of the blood was centrifuged at 3000 rpm to obtain the blood serum for determining the contents of triglyceride, cholesterol, and high-density lipoprotein (HDL). The rats were killed by disjoint methods, and the body weight and body height were determined, so the Lee index could be calculated as body weight (g) × 10^3/body height (cm). Groin subcutaneous fat, abdominal cavity and intestines fat, and fat around the kidneys were removed and weighed. The fat cell size was observed, and fat cell number was counted under a microscope (*14*).

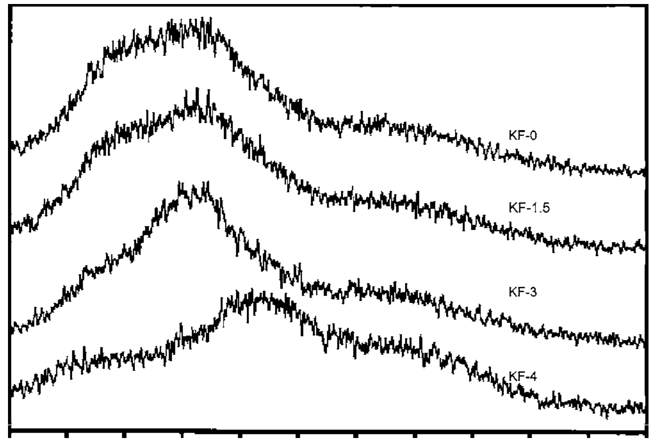

Figure 1. X-ray diffraction patterns of native konjac flour and ball-milled konjac flour.

RESULTS

Granularity. The d_{50} values of KF-0, KF-1.5, KF-3, and KF-4 were 657.3, 487.9, 140.5, and 23.7 μm, respectively, and the dispersion of distribution increased visibly. The grain size did not change very much in the first 1.5 h. However, after 3 h of ball-mill treatment, the grain size decreased sharply and ulteriorly in the following 1 h.

Crystallinity. Native konjac flour is a kind of polysaccharide having relatively low crystallinity, so it presented itself as amorphous. It had been reported that the flexibility of micronized starch, prepared by physical methods, increased due to strong mechanical stress. The same result had been drawn from XRD patterns of the native konjac flour and ball-milled konjac flour (**Figure 1**). The crystallinity of native konjac flour was 36.56%, and the crystallinities of KF-1.5, KF-3, and KF-4 were 34.46, 30.06, and 13.26%, respectively, which indicted that the flexibility of KF-4 was the greatest. Such a result answered for the results of granularity well. In the first 1.5 h, which was mainly surface pulverization (*15*), the grain size of konjac flour changed minutely, and the crystallinity decreased unnoticeably. When the treatment time was 3 h, the grain size change of konjac flour was relatively large. In the last 1 h treatment, the grain size of konjac flour changed significantly, and the crystallinity decreased sharply; at the same time the position of the diffraction peak drifted, so perhaps structural changes had taken place. Because the KGM was a kind of heteropolysaccharide, some bonds could be broken more easily; however, some bonds were difficult to break (*16*).

Thermal Properties. There are two kinds of crystals in konjac flour: one is a KGM−water crystallite, the other is a KGM−KGM crystallite; both are formed by hydrogen bonds. The DSC curves of konjac flour and ball-milled konjac flour showed that compared with native konjac flour the thermal properties of ball-milled konjac flour varied greatly. The thermal properties of konjac flour and ball-milled konjac flour are listed in **Tables 1** and **2**; the temperatures in **Tables 1** and **2** indicate the melting temperatures of KGM−water crystallites and KGM−KGM crystallites, respectively (*17*). With the size of konjac flour granules decreasing, all of the temperature parameters of konjac flour−water crystallites changed haphazardly expect the enthalpy (ΔH), which decreased in an orderly fashion, which indicated that the KGM−water crystals could be broken by mechanical micronization mainly by reducing the crystallization region rather than changing the crystalline structure (*17*). The T_{P2}, T_{C2}, T_{E2}, and ΔH_2 of KGM−KGM crystallites decreased in an orderly manner with increasing treatment time,

Table 1. Thermal Properties a of Native Konjac Flour and Ball-Milled Konjac Flour (Peak 1)

sample	onset temp (T_{O1}, °C)	peak temp (T_{P1}, °C)	conclusion temp (T_{C1}, °C)	extrapol peak temp (T_{E1}, °C)	enthalpy (ΔH, J g^{-1})
KF-0	33.61	63.82	100.98	48.49	−263.41
KF-1.5	32.99	65.67	104.19	42.55	−232.57
KF-3	33.60	65.84	96.90	60.50	−189.75
KF-4	33.25	63.21	90.26	40.57	−125.39

Table 2. Thermal Properties a of Native Konjac Flour and Ball-Milled Konjac Flour (Peak 2)

sample	onset temp (T_{O2}, °C)	peak temp (T_{P2}, °C)	conclusion temp (T_{C2}, °C)	extrapol peak temp (T_{E2}, °C)	enthalpy (ΔH_2, J g^{-1})
KF-0	293.06	323.37	338.52	325.83	−189.94
KF-1.5	293.09	322.04	337.42	324.75	−182.14
KF-3	292.75	318.89	334.20	321.08	−133.73
KF-4	297.58	318.63	331.87	320.75	−63.57

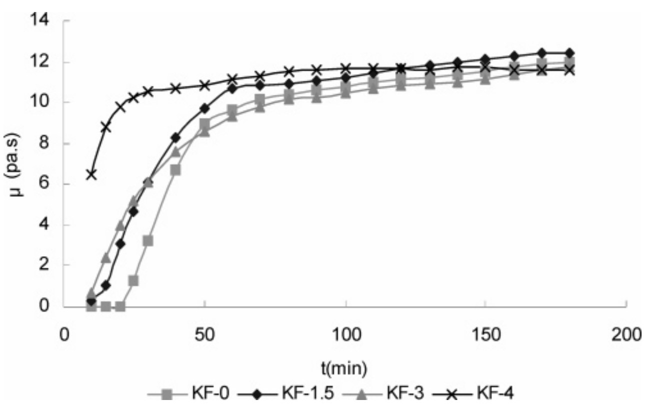

Figure 2. Swelling velocity of native konjac flour and ball-milled konjac flour.

which also indicated that the KGM−KGM crystals could be broken by ball-mill treatment, not only by reducing the crystallization region but also by changing the crystalline structure (*18*). This result was the same as that of XRD.

Swelling Velocity. The swelling velocity of konjac flour with different grain sizes is shown in **Figure 2**; the results show that all of the samples after ball-mill treatment swelled more rapidly, especially in KF-4, which took only 30 min to achieve the highest viscosity, whereas the native konjac flour took at least 60 min. Such a result could be explained without difficulty by the grain-size change and the crystallinity change above. With decreasing grain size and crystallinity, the konjac flour grain could be drenched by water more easily. As for KF-4, the reason for its unusual swelling might be connected with the drift of the diffraction peak as well as the changes of structure.

Antiobesity Character. *Effect on Growth of Nutritional Obese Rats.* The effect of ball-milled konjac flour on the growth of nutritional obese rats is shown in **Table 3**; the initial average body weight was 52.7±2.5 g for all six groups. The average body weight of the nutritional obese group increased rapidly and was twice that of the control group with the basal feed and had a significant difference ($P < 0.01$), which indicted that the nutritional obese model was successful. The average body weight of the native konjac flour group (KF-0) increased by slow degrees, and both groups had significant difference versus the nutritional obese rat group ($P < 0.05$); the average body weight was higher than that of the control group. However, the average body weight of the ball-milled konjac flour (KF-4) group increased most slowly and not only had a significant difference versus the nutritional obese rats group ($P < 0.01$)—the average body weight was lower than that of the control group—but also had a significant difference versus the native konjac flour group ($P < 0.05$). Therefore, it could be concluded that the ball-milled konjac flour had a better antiobesity effect than the native konjac flour.

Table 3. Effect of Ball-Milled Konjac Flour on the Growth of Nutritional Obese Rats ($\bar{x} \pm s$, $n = 12$)

group	dosage (g·kg^{-1})	feed sort	initial av body wt (g)	final av body wt[a] (g)	Lee's index[b]
control		basal	52.7 ± 2.5	66.2 ± 29.2**	301.4 ± 16.9
obesity model		nutritious	52.9 ± 2.4	123.1 ± 21.5	308.2 ± 17.9
KF-0	0.8	nutritious	53.2 ± 2.6	88.9 ± 24.1*	304.7 ± 11.8
	0.2	nutritious	52.4 ± 2.5	86.5 ± 22.2*	301.9 ± 12.2
KF-4	0.8	nutritious	53.3 ± 2.7	62.3 ± 20.8**#	309.6 ± 14.1
	0.2	nutritious	52.2 ± 2.3	62.0 ± 20.4**#	308.1 ± 13.7

[a] *, $P < 0.05$; **, $P < 0.01$, versus nutritional obese rats group; #, $P < 0.05$, versus native konjac flour (KF-0) group. [b] Lee's index = body weight (g) × 10^3/body height (cm).

Table 4. Effect of Ball-Milled Konjac Flour on Parameter in Weight Loss of Nutritional Obese Rats ($\bar{x} \pm s$, $n = 12$)

group	dosage (g·kg^{-1})	total wet wt of fat[a] (g)	fat cell no.[a]	fat cell size[a] (μm)
control		2.53 ± 1.63**	93 ± 11**	50.5 ± 4.2**
obesity model		7.29 ± 2.23	36 ± 8	94.8 ± 7.1
KF-0	0.8	4.46 ± 1.32	58 ± 7**	61.9 ± 4.1**
	0.2	4.18 ± 1.36	64 ± 8**	63.5 ± 4.5**
KF-4	0.8	2.93 ± 1.43**#	57 ± 8**	57.3 ± 4.8**
	0.2	2.86 ± 1.44**#	58 ± 7**	58.2 ± 5.4**

[a] *, $P < 0.05$; **, $P < 0.01$, versus nutritional obese rats group. #, $P < 0.05$, versus native konjac flour (KF-0) group.

Effect on Parameter in Weight Loss of Nutritional Obese rats. The effect of ball-milled konjac flour on parameter in weight loss of nutritional obese rats is shown in **Table 4**. The fat cell size of all the groups except the nutritional obese group decreased significantly ($P < 0.01$) versus the nutritional obese rats group, whereas there was no significant difference in these groups. The result of the fat cell number was similar to that of the fat cell size. The native konjac flour group (KF-0) could decrease the total wet weight of fat; both groups had significant difference versus the nutritional obese group ($P < 0.05$), and the total wet weight of fat was higher than that of the control group, which had a significant difference versus the nutritional obese group ($P < 0.01$). However, the ball-milled konjac flour (KF-4) group not only had a significant difference versus the nutritional obese group ($P < 0.01$) and the average body weight was near that of the control group, but also there was a significant difference versus the native konjac flour group ($P < 0.05$). This also indicated that the ball-milled konjac flour had the better antiobesity effect.

Effect on Blood Fat and Blood Glucose of Nutritional Obese Rats. The effect of ball-milled konjac flour on blood fat and blood glucose of nutritional obese rats is shown in **Table 5**. The fat cell size of all the groups had no significant difference. The triglyceride and blood glucose values of both the native konjac flour (KF-0) and the ball-milled konjac flour (KF-4) group decreased significantly ($P < 0.05$), and the ball-milled konjac flour group decreased more ($P < 0.01$). The HDL of control and the ball-milled konjac flour group decreased

Table 5. Effect of Ball-Milled Konjac Flour on Blood Fat and Blood Sugar of Nutritional Obese Rats ($\bar{x} \pm s$, $n = 12$)

group	dosage ($g \cdot kg^{-1}$)	cholesterol ($mmol \cdot L^{-1}$)	triglyceride[a] ($mmol \cdot L^{-1}$)	HDL[a] ($mmol \cdot L^{-1}$)	blood sugar[a] ($mmol \cdot L^{-1}$)
control		2.4 ± 0.3	0.73 ± 0.11*	0.58 ± 0.12**	3.4 ± 1.9**
obesity model		2.3 ± 0.4	1.16 ± 0.49	0.73 ± 0.15	5.6 ± 0.8
KF-0	0.8	2.3 ± 0.3	0.58 ± 0.07*	0.62 ± 0.21	4.1 ± 1.2*
	0.2	2.4 ± 0.3	0.60 ± 0.12*	0.65 ± 0.29	4.2 ± 1.6*
KF-4	0.8	2.0 ± 0.4	0.47 ± 0.42**	0.52 ± 0.20**	3.2 ± 1.8**
	0.2	2.0 ± 0.3	0.49 ± 0.33**#	0.54 ± 0.18**#	3.3 ± 1.3**#

[a] *, $P < 0.05$; **, $P < 0.01$, versus nutritional obese rats group; #, $P < 0.05$, versus native konjac flour (KF-0) group.

significantly versus the nutritional obese group ($P < 0.01$). However, the native konjac flour group had no evident difference with the nutritional obese model group.

It is well-known that konjac flour is a kind of good-quality water-soluble diet fiber; after absorbing the water, the konjac flour swells and turns into jelly. The konjac jelly adheres to the small intestine mucous membrane and forms a kind of separator, which decreases the absorption of glucose in the small intestine. The reason the blood glucose level of the KF-4 group decreased more might be that it could swell more expeditiously and the separator could form instantly. In addition, KF-4 could stay longer in the stomach and intestines compared with the native konjac flour due to its swelling velocity, so it might bind to bile acids in the gut and carry them out of the body in the feces, which requires the body to convert more cholesterol into bile acids.

Conclusion. The grain size changed from 657.3 μm (d_{50}) to 23.7 μm (d_{50}) after 4 h of treatment, and the pulverized model of konjac flour seemed to be a mixture of surface-pulverized model and volume-pulverized model, but was mainly the latter.

The ball-mill treatment not only resulted in the decrease of the grain size but also brought about the grain-size effect on the structure and antiobesity bioactivity. The crystallinity decreased obviously and presented itself as a drift of diffraction peak when treated for 4 h; the thermal character of the konjac flour also changed at the same time. Such changes of structure due to the grain-size effect endowed the changes of swelling velocity of the konjac flour. That the KF-4 could swell much more rapidly would be helpful for its antiobesity activity.

Compared with native konjac flour, the KF-4 could significantly decrease the rat body weight and the Lee coefficient of nutritional obese rats ($P < 0.05$) and significantly decrease the contents of triglyceride, glucose, cholesterol, and HDL in the blood of the nutritional obese rat ($P < 0.05$). The mechanism might be that it could delay stomach emptying, making a person feel full, and lead to a more gradual absorption of dietary sugar; the konjac could bind to bile acids in the gut and carry them out of the body in the feces, decreasing the cholesterol content due to the outstanding swelling velocity developed from the grain-size effect of konjac flour.

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