Research Article

Received: 30 June 2009 Revised: 20 November 2009 Accepted: 23 November 2009 Published online in Wiley Interscience: 1 February 2010

(www.interscience.wiley.com) DOI 10.1002/jsfa.3870

The effects of steaming and roasting treatments on β-glucan, lipid and starch in the kernels of naked oat (*Avena nuda*)

Xinzhong Hu,[a]* Xiaohui Xing[a] and Changzhong Ren[b]

Abstract

BACKGROUND: Normal pressure steaming (NPS), autoclaved steaming (AS), and hot-air roasting (HAR) are widely used to deactivate oat enzyme in the oat-processing industry. Infrared roasting (IR) is a new oat deactivation method, and is welcomed and employed by increasing numbers of oat-processing plants in China. It is widely known that oat starch plays an important role in the processing function of oat food, and that oat β-glucan and lipid contribute greatly to the health benefits of oat food. However, the effects of steaming and roasting treatments on the starch, β-glucan and lipid in oat kernels are poorly known.

RESULTS: In this research, the level and distribution of β-glucan and lipid in oat kernels with and without deactivation treatments were tested. We also measured the viscosity properties of oat flour from kernels after NPS, AS, HAR and IR treatments, and examined the effects of these treatments on oat starch granularity using scanning electron microscopy. The results showed that the deactivation treatments did not have significant effects on oat β-glucan and lipid levels in oat kernels ($P < 0.01$). The distribution of β-glucan and lipid in enzyme-deactivated kernels was very similar to that in normal kernels. NPS, AS, HAR and IR treatments changed the shape of starch granules, crumbled large starch granules, reduced the connection between the protein network and starch granules, and improved starch gelatinization properties.

CONCLUSIONS: NPS, AS, HAR and IR treatments can change the structure of oat starch granules and improve the viscosity property of oat starch without causing β-glucan and lipid loss to oat food.

Keywords: naked oat (*Avena nuda*); enzyme deactivation; infrared roasting; starch; β-glucan; lipid

INTRODUCTION

Oat kernel is rich in lipase and lipoxidase, so treatments to deactivate its enzymes are essential for the processing and storage of oat food.[1–4] Hot-air roasting (HAR) and steaming, including normal pressure steaming (NPS) and autoclaved steaming (AS), are commonly used in China as oat enzyme deactivation treatments. Owing to the wide use of these treatments, it is important to study their effects on oat nutrition and processing. However, because these treatments are time-consuming and fail to fit the industrialization process in large oat-processing companies in China, researchers and processors have been searching for a new deactivation method for oat processing.[5,6] Infrared roasting (IR) is a novel deactivation treatment in China, and its influence on the nutritious value of oat and its processing property is generating new research interests.

The structure and physical properties of starch correlate highly with the processing and palatability of cereal food. Many have reported that high temperature damages the hydrogen bonds and crystal regions of starch granules, resulting in use of a low temperature for gelatinization initialization.[7] Gonzalez *et al*.[8] reported that a roasting treatment significantly lowered the crystallinity and gelatinization enthalpy of alfalfa starch. Oat starch constitutes 54.9–63.6% of a kernel's weight.[7] However, there are no reports concerning the effects of steaming and roasting on the structure and physical properties of oat starch. In the current study we used the starch staining method and scanning electronic microscopy (SEM) to observe starch distribution and the structure of oat kernels with or without deactivation treatments, with the intention of ascertaining whether or not NPS, AS, HAR and IR treatments have significant effects on oat-processing properties.

It is widely accepted that β-glucan is an important nutritional ingredient in oat kernel. Numerous clinical studies have shown that the consumption of oats or oat bran lowers serum cholesterol in individuals with elevated serum.[9–12]. Lipid is also important for oat nutrition. The oat antioxidant property is attributed to the oat lipid compound, which is rich in polyunsaturated acid, and in particular oleic acid and linoleic acid.[6] In the present study, we also intended to examine whether or not there were remarkable β-glucan or lipid-level changes caused by steaming and roasting treatments. This is important because oat β-glucan and lipid contribute greatly to the nutritious value of oat food, and any loss of oat β-glucan

* *Correspondence to: Xinzhong Hu, College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China. E-mail: hxinzhong@yahoo.com*

a *College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China*

b *Baicheng Academy of Agricultural Science, Baicheng, Jilin 137000, China*

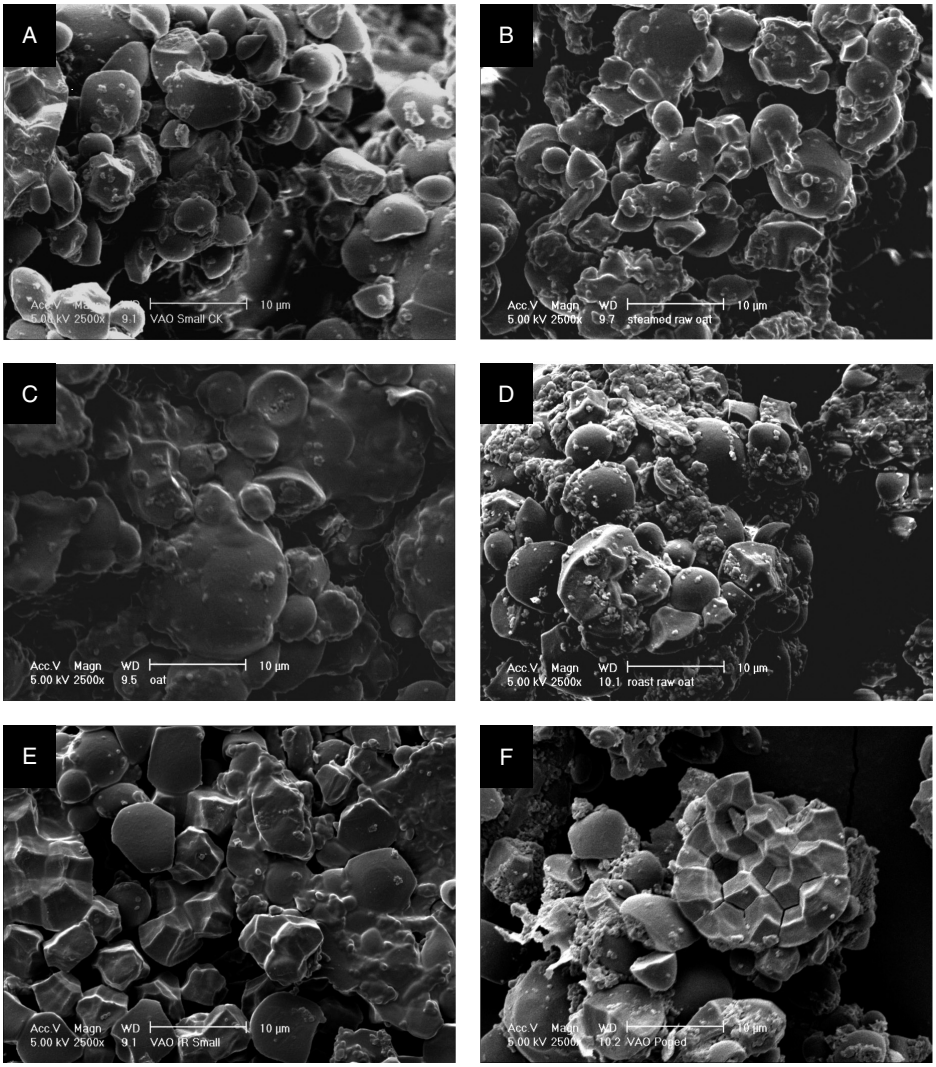

Figure 1. SEM pictures of oat kernels with and without deactivation treatments: (A) control; (B) NPS treatment; (C) AS treatment; (D) HAR treatment; (E) IR treatment; (F) puffed kernel. × 2500. The picture for each group was chosen at random from 40 images in this group.

and lipid by deactivation treatment is undesirable. To the best of our knowledge, this is the first ever report on the role of enzyme deactivation treatments on the levels of oat β-glucan and lipid.

EXPERIMENTAL

Materials

VAO-2 naked oat kernels were harvested in 2005 from a 15 ha field in Ottawa (Ontario, Canada). The oat kernels were cleaned, dehulled and purified, and then graded with a dockage tester (5/64 × 3/4). Kernels of a large size (left in the upper side) and similar shape were chosen for lipase deactivation treatments, since our previous research found kernel size and shape had a great effect on lipase activity.[5]

Methods

Enzyme activities were deactivated by four different treatments described as follows.

Normal pressure steaming (NPS) was implemented following Ames and Rhymer's methods.[13] Oat kernels were placed into the metal basket of a vegetable steamer and treated over boiling water for 20 min. The kernels were steamed in batches of 250 g, which allowed the kernels to form a layer no deeper than 1 cm in the steamer basket. After NPS treatment, the kernels were kept at room temperature for 24 h for moisture equilibration. They were then put in an air oven at 33 °C for 12 h to reduce the moisture content to about 10%.

High pressure steaming (AS) was implemented according to Zhang *et al.*'s report[14] and modified slightly. Using an autoclave, oat kernels were steamed at 121 °C and 15 psi for 10 min. The samples were then exposed at room temperature for 24 h to allow moisture equilibration, and then kept in an air oven at 33 °C for 12 h to reduce the moisture content to about 10%.

Hot air roasting (HAR) was performed following Zhang *et al.*'s report.[14] Before the roasting treatment, oat kernels were tempered for 3 h to a moisture content of 20%. The tempered oat kernels were then roasted in a hot-air oven at 155 °C for 30 min. The HAR-treated kernels were then kept in an air oven at 33 °C for 12 h, which allowed the moisture content to drop to about 10%.

Infrared roasting (IR) was implemented using a BO-04 infrared instrument (Micronizing Company UK Ltd, Charnwood Mill, Framlingham, UK). Tempered oat samples with a moisture content

Table 1. Peak viscosity, trough, breakdown, final viscosity, setback and starch content with or without deactivation treatments

Deactivation treatment	Peak viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Starch content (%)
Control	240.9 ± 0.6d	153.8 ± 0.4e	87.1 ± 0.3a	466.7 ± 4.8a	397.5 ± 4.9a	56.4 ± 0.3a
NPS	309.0 ± 2.3a	246.3 ± 3.5a	62.8 ± 1.4c	425.2 ± 4.5c	178.8 ± 1.0e	49.9 ± 3.2a
AS	280.1 ± 0.4b	225.8 ± 0.2b	54.3 ± 0.2d	418.8 ± 0.5c	192.9 ± 0.4d	50.1 ± 0.5a
HAR	241.5 ± 2.8d	165.6 ± 1.0d	75.9 ± 1.9b	449.5 ± 0.1b	283.8 ± 1.0b	56.3 ± 2.4a
IR	261.2 ± 2.1c	204.6 ± 1.0c	56.6 ± 0.4cd	404.8 ± 1.2d	200.2 ± 2.2c	54.3 ± 0.2a

Means in a column followed by different letters are significantly different at $P < 0.01$.

Table 2. Lipid content, lipase activity, peroxidase activity and β-glucan content of oat kernels with or without deactivation treatment

Deactivation treatment	β-glucan content (%)	Lipid content (%)	Lipase activity (μmol g^{-1} h^{-1})	Peroxidase activity
Control	4.23 ± 0.14a	6.29 ± 0.04a	220 ± 2.1	+
NPS	3.30 ± 0.05a	6.19 ± 0.09a	–	–
AS	4.02 ± 0.20a	6.18 ± 0.21a	–	–
HAR	3.56 ± 0.35a	6.76 ± 0.07a	39 ± 1.7	+
IR	3.95 ± 0.33a	6.15 ± 0.09a	–	–

Means in a column followed by different letters are significantly different at $P < 0.01$. '+' means peroxidase activity detected; '–' means peroxidase activity or lipase activity not detected.

of 20% were infrared-roasted at 580 °C for 18 s. After IR treatment, the moisture content of the oat kernels was adjusted to about 10% by keeping the kernels in an air oven at 33 °C for 12 h.

The levels and distribution of the β-glucan, lipid, and starch in the kernels with or without enzyme deactivation treatments were examined according to the following methods.

Scanning electron microscopy (SEM): Each oat kernel was cut transversely into slices of 1 mm thick using a sharp-edged blade, soaked in fixing solution (3% glutaraldehyde solution) for 12 h, and then soaked in phosphate buffer (PB, 0.1 mol L^{-1}, pH 7.0) for 15 min. Samples were dehydrated consecutively in ethanol solvents with ascending concentrations of 15%, 30%, 50% and 70%, with 15 min allocated for each treatment. They were further dehydrated consecutively in 80%, 90% and 95% ethanol solvents, with 20 min for each treatment, and were then dehydrated in 100% ethanol for 1 h. Next, the dehydrated samples were soaked in a 100% isoamyl acetate bottle for 1 h, and then dried using a CO_2 critical point drier (EMITECH K850, Ashford, UK). The dried samples were fixed on a copper stub using double adhesive carbon conductive tape and coated with platinum for 30 s using a platinum coating facility (Auto Fine Coater JFC-1300, Jeol, Tokyo, Japan). Samples were then observed and photographed on a scanning electron microscope (JSM-6360LV, Jeol).

The starch content in oat flour, viscosity properties of oat flour, β-glucan content, kernel lipid content, and peroxidase activity of the oat samples were tested according to AACC 76-13, AACC 76-22, AACC 31-22, AACC 30-10, and AACC 22–80, respectively. Lipase activity was determined according to Kwon and Rhee's reports.[15] Oat kernels were powdered and defatted, and 0.5 g defatted oat powder was mixed with 98 µL triolein. The mixture was added to 330 µL buffer (0.05 mol L^{-1} Tris-HCl, pH 7.5, containing 1% v/v Triton X-100) and stirred to form oat dough. The dough was incubated at 37 °C for 1 h, and then 100 µL 1 mol L^{-1} HCl was added to stop the incubation. HCl was then added immediately as a zero-time control. The dough was soaked and boiled in 5 mL isooctane extractant for 300 s to extract oleic acid, which was the product of triolein hydrolysis. The absorbance of the extract was then measured at 715 nm, and was further compared with the absorbance from an oleic acid standard. Lipase activity was described in units of μmoles of oleic acid per hour per gram.

β-Glucan microscopy:[16] Each oat kernel was soaked in distilled water for 2 days, and then soaked consecutively in cellosolve, 95% ethanol, *n*-propyl alcohol, and *tert*-butyl alcohol, with a soaking time of 24 h in each organic solvent. Next, each oat sample was transversely cut into slices of 3 µm thick using an MT-1 microtome (Sorvall Porter Blum, Ivan Sorvall Inc., Newtown, CT, USA). Each oat slice was then placed on a microscope slide. To fix the oat slice on the microscope, a drop of water was placed on the slide, allowed to spread over the slide surface, and then evaporated at 50 °C in a fan-blast drying oven. The oat slice was then stained blue with several drops of 0.01% Calcofluor White M2R New for 60 s, washed with distilled water after the staining, treated with 0.01% acidic fuchsine for 60 s, and then washed with distilled water again. Any water drops left on the slides were blown away by compressed air. All the slices were then observed and photographed on a Carl Zeiss HBO 100 fluorescent microscope (Carl Zeiss Vision GmbH, Hallbergmoos, Germany).

Lipid microscopy:[16]

The oat kernels were soaked in distilled water for 24 h, and then cut into small pieces and soaked in a potassium phosphate buffer (containing 6% glyceraldehyde and 0.025 mol L^{-1} potassium phosphate) for 2 days. They were then frozen and buried quickly with a freezing solvent. The frozen oat samples were then cut transversely into slices of 10 µm thick at −22 °C using a freezing microtome. Each slice was placed on a microscope slide, stained with 0.01% Nile Blue, gently covered with a cover-glass, and observed and photographed using a Carl Zeiss HBO 100 fluorescent microscope.

Statistical analysis

Values are given as means ± standard division (SD). The statistical significance of the differences among the parameters was assessed using analysis of covariance (ANOVA) by SAS software, and group means were considered to be significantly different at $P < 0.01$.

RESULTS AND DISCUSSION

Effects of steaming and roasting on starch in oat kernels

SEM results demonstrated that the cobblestone-liked shape of starch granules in the control kernels was very clear, with a few

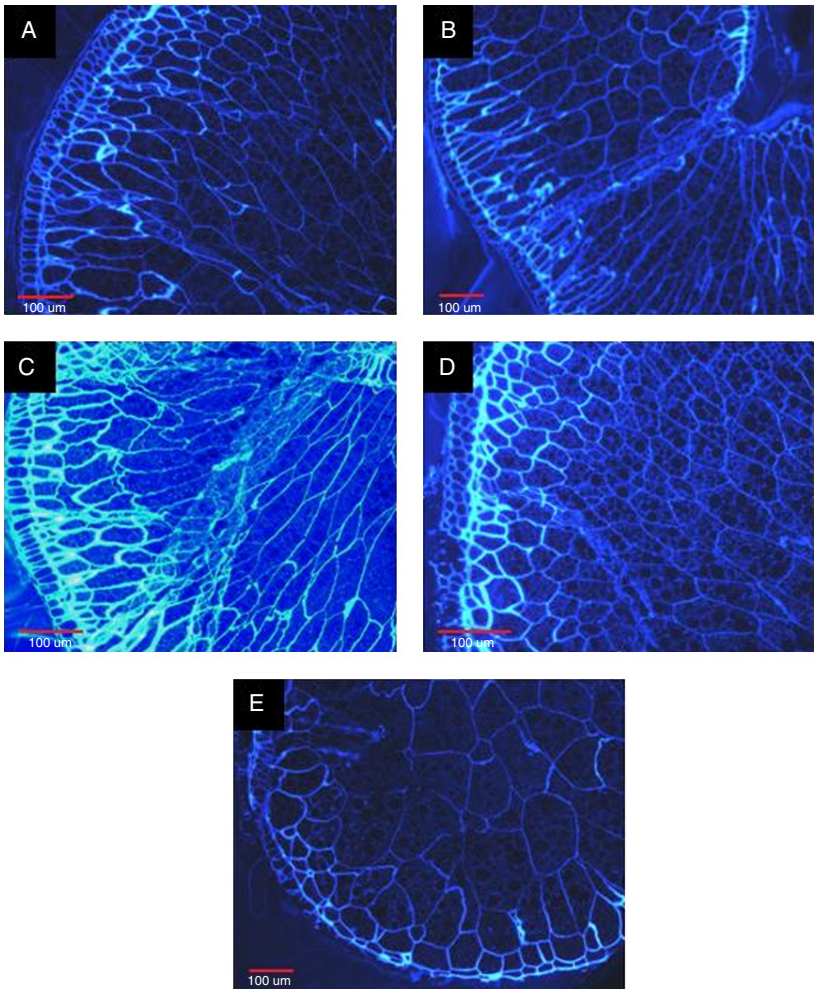

Figure 2. β-Glucan distribution in oat kernels with and without deactivation treatments: (A) control; (B) NPS treatment; (C) AS treatment; (D) HAR treatment; (E) IR treatment. The picture for each group was chosen at random from 40 images in this group. The scale line in each of the pictures stands for 100 μm.

large starch granules surrounded by many small ones (Fig. 1(A)). Floccular oat protein networks attached closely to the surface of oat starch granules could be easily observed in control kernels. During NPS treatment, it was hard to see cobblestone-shaped starch granules and floccular protein networks (Fig. 1(B)). The NPS treatment decomposed large starch granules into small pieces, which adhered to each other to form irregular shapes. The adhesion and transformation of starch granules and disappearance of the protein network were also observed in AS-treated kernels (Fig. 1(C)). Compared with NPS treatment, AS treatment resulted in a more compact kernel structure. In HAR treatment, cobblestone-shaped starch granules and protein networks were recognizable, but large starch granules were very rare, while protein networks were very loose (Fig. 1(D)). During IR treatment, due to the thermo-effect of infrared radiation, large starch balls disintegrated to form numerous small multi-angular starch pieces, with limited numbers of large starch balls remaining (Fig. 1(E)). Interestingly, the surface of the partly disintegrated large starch ball was much like the surface of a football. We further compared the IR pictures with a previously unpublished puffed oat picture in our lab. We noticed that the starch ball structure in the IR-treated oat was similar to that in a puffed oat (Fig. 1(F)). There were also some large starch granules with interesting 'football' surfaces observed in the puffing treatment. The above results indicated that deactivation treatments could change the shape of starch granules, crumble large starch granules, and reduce the connection between protein network and starch granules. The crumbling, adhesion, and transformation of starch balls imply that deactivation treatments might pre-gelatinize oat starch. This further gave us indication that deactivation treatments could have significant effects on the starch gelatinization property of oat food, which was confirmed by the results of the viscosity test of oat flour. The peak viscosities and troughs of the NPS, AS and IR groups were significantly higher ($P < 0.01$) than for the control group, whereas the breakdown, final viscosity and setback levels in any of the activation treatments were significantly lower ($P < 0.01$) than in the control group (Table 1). The above results confirmed Sakonidou *et al.*'s report[17] that heating treatments could break the hydrogen bonds connecting starch molecules, discompose starch granules, and result in starch gelatinization.

Effects of steaming and roasting on β-glucan and lipid in oat kernels

Many researchers have reported that the cholesterol-lowering and anti-atherogenic activity of oat or oat bran are largely attributable to the β-glucan component.[18] In this study, we paid special

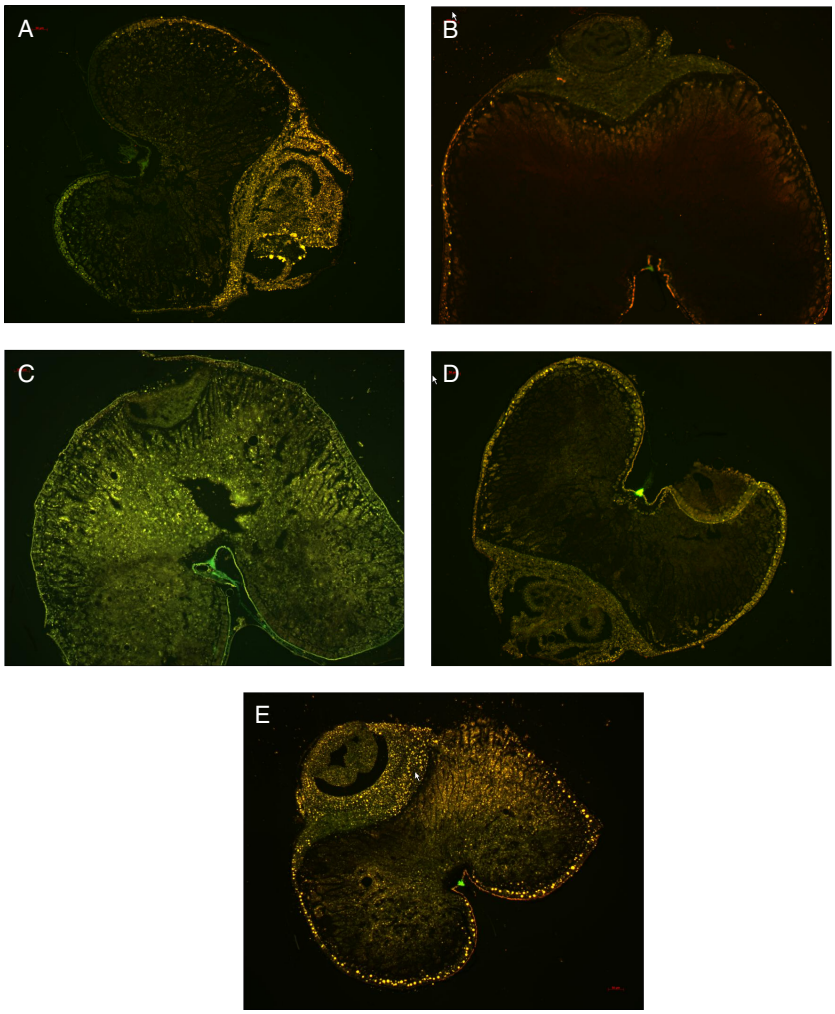

Figure 3. Lipid distribution in oat kernels with and without deactivation treatments: (A) control; (B) NPS treatment; (C) AS treatment; (D) HAR treatment; (E) IR treatment. The picture for each group was chosen at random from 40 images in this group.

attention to the effects of different deactivation treatments on the oat β-glucan content in oat kernels, because the actual concentration of β-glucan is very small in oat kernel ($<5\%$)[19,20] and a small loss of β-glucan during food processing could result in a large reduction in its health benefits. The results showed that there were no significant differences in the β-glucan content in oat kernels among NPS, AS, HAR, IR and control groups (Table 2). In this study, we did not observe any significant difference in lipid levels between the control groups and any of the deactivation treatment groups (Table 2). The above results indicated that NPS, AS, HAR and IR treatments to VAO-2 naked kernels did not cause significant nutritional losses to oat kernels in terms of oat β-glucan or lipid content. We also observed in the present study that NPS, AS and IR treatments could efficiently exterminate lipase and peroxidase activities (Table 2). Interestingly, in another study carried out in our lab, the same parameters used in the NPS, AS, HAR and IR treatments in the present study were also used to treat the kernels of VAO-3 and VAO-10, another two Canadian oat varieties. The results showed that NPS and AS treatments could deactivate lipase activities in these kernels completely, while the IR treatments failed to completely deactivate the lipase activities in the kernels of these two oat varieties. Instead, 4 and 14 μmol g^{-1} h^{-1} remained in VAO-3 and VAO-10 oat kernels, respectively (unpublished data). Thus the effects of IR treatments on different oat varieties are variable, and the results from the VAO-2 oat in the present study do not disconfirm that peeling treatment is necessary for some oat varieties.

We also demonstrated the distribution of β-glucan and lipid in normal oat kernels and kernels with NPS, AS, HAR and IR treatments (Figs 2 and 3). The results showed that β-glucan and lipid levels in the outer layer of normal oat kernels were higher than those inside the kernel. Similar to normal kernels, the difference between the kernel's outer layer and its inside in terms of β-glucan and lipid levels still exists in NPS, AS, HAR and IR treatments. The results were meaningful for oat peeling treatment, which was described in our previous study.[5] It is easy to see from Fig. 2 that β-glucan constituted the cell wall of the oat cell and that there was a considerable amount of β-glucan in the outer layer of the kernel. What should be mentioned was that the oat β-glucan was not distributed evenly in the kernel outer layer. Low fluorescent light in the cortex indicated that the β-glucan level in the cortex was very limited, while high fluorescent light in the aleurone layer implied richness in β-glucan. This result confirmed our previous conclusion that peeling treatment should not exceed 20 s, because otherwise the treatment could damage the β-glucan-rich aleurone layer and result in great β-glucan loss.[5]

Advantage and perspective of IR deactivation treatment

With shorter treatment time than other enzyme deactivation methods, IR treatments can greatly reduce lipase and peroxidase activity without great β-glucan or lipid loss, and cause remarkable starch gelatinization, which is helpful for oat processing. However, the effects of IR treatment on different oat varieties are variable. In fact, for many oat varieties, kernels after IR treatment still have residual lipase activities, and thus appropriate pretreatments to remove part of the lipase activity from these kernels before IR treatment are necessary. Our previous report introduced the 'oat rice'.[5] 'Oat rice', i.e. oat kernels that have had peeling treatments before enzyme deactivation, is a novel oat product in China. Compared to normal oat kernels, 'oat rice' has much lower lipase activity, and for this reason it should be more suitable for IR treatment. What should be mentioned is that 'oat rice' can be added to rice and cooked with it, and so it accords well with the eating habits of Chinese people. The potential market demand for 'oat rice' is huge and as such has stimulated much interest for oat processors and researchers in China. We therefore suggest that further study should be implemented on the effect of IR treatment on 'oat rice'.

CONCLUSIONS

NPS, AS, HAR and IR treatments did not cause significant loss of β-glucan or lipid content in oat kernels. All four treatments changed the shape of starch granules, crumbled large starch granules, reduced the connection between protein networks and starch granules, and improved starch gelatinization properties, implying that deactivation treatments were helpful for oat food processing. Future research should be carried out on IR treatment, which has a much shorter treatment time compared to NPS, AS and HAR treatments.

ACKNOWLEDGEMENTS

We thank Dr Judith Frégeau-Reid, Dr Jian-Qiang Zhou, Dr Vernon D. Burrows, Ms Louise Florent and Ms Eglanten for giving us support and encouragement to carry out this work. We also gratefully acknowledge Dr Todd Smith for making grammatical corrections to this paper. This research was financially supported by the Public Industry Project from the Ministry of Agriculture (nyhyzx07-009), the Northwest A&F University Key Research Project for the Young Staff and Agriculture Development Project from the Yangling Demonstration Zone, and the earmarked fund for modern Agro-industry Technology Research System (nycytx-014).

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