Prev. Nutr. Food Sci. 2020;25(1):50-57
https://doi.org/10.3746/pnf.2020.25.1.50
pISSN 2287-1098 · eISSN 2287-8602

Acidic-Treated Acorn Pollen as Health Functional Food Materials for Improvement of Post-Menopausal Glucose Metabolism

Su Jin Nam[1], Soo Im Chung[2], and Mi Young Kang[1]

[1]Department of Food Sciences and Nutrition and [2]International Agricultural Training Center, Kyungpook National University, Daegu 41566, Korea

ABSTRACT: Pollen has high physiological value because it contains protein, essential amino acids, and 16 vitamins. However, pollen is difficult to absorb because of its hard form. This study explores the use of the acid-treated acorn pollen (acorn pollen deposited in apple vinegar for 30 days). The health functions of acid-treated acorn pollen on post-menopausal metabolism was tested by analyzing *in vitro* and *in vivo* biomarkers for glucose metabolism, by using the acid-treated acorn pollen and its residues, respectively. *In vitro* experiments showed high activity after measuring the low potency of glucose-related enzymes. *In vivo* experiments showed reduced blood glucose and insulin levels after consuming pollen. Pollen also increased the concentration of glucokinase, a glucose-regulating enzyme in hepatic and nephritic tissue, and lowered the concentration of glucose-6-phosphatase. These results are encouraging in showing that acid pollen can be used as a functional health food for treatment of post-menopausal metabolism.

Keywords: acidic-treated, acorn pollen, amino acid, enzyme inhibition, glucose metabolism

INTRODUCTION

Pollen is a folk remedy known as "stamina, health enhancement, enhancement of immunity, menopause, and good food for women's skin". Pollen is the main ingredient of Royal Jelly and contains protein, essential amino acids, and 16 vitamins, etc. (Chung et al., 1984). Pollen is a high-degree nutritional food containing 41 different nutrients required by humans need a daily basis (Kim et al., 1984). In addition, oriental medicine has reported that the nutritional value of pollen has a high physiological value, since it shows beneficial effects against neurological disorders, atherosclerosis, and geriatric diseases (Fang et al., 2008; Li et al., 2009; Abouda et al., 2011). From this point of view, a beekeeper is worth considering for healthy food materials as a way to increase income other than honey. In fact, honey is used as a dietary supplement in USA, as a medicinal product in Germany (Kroyer and Hegedus, 2001; Llnskens and Jorde, 2001), and as a health supplement in Korea. Pollen is made up of hard exine and intine. Therefore, when most animals and insects eat pollen, they cannot break it down and only absorb some nutrients (Roulston and Cane, 2000; Blackmore et al., 2010). Pollen is the substance containing a large amount of health-functioning compounds (Andradea et al., 1997), and its use as a beneficial health product should be considered. Therefore, methods for grinding, erosion, and decomposition of the surface of pollen (Kress et al., 1978; Lee, 1986; Kim and Son, 1990; Lee et al., 1997; Choi and Jeong, 2004), and methods to leach ingredients inside the pollen (Suk and Kim, 1983; Kim et al., 1992) have been used (Kim, 1989). However, pollen is still consumed in the form of pollen. Hong et al. (2014) confirmed that there is a hole in the surface of pollen, and Lee et al. (1997) reported a study in which the constituents of pollen were extracted with hexane and water through the hole in the surface. This study was designed to study the supernatants and precipitations of pollen extracts by depositing pollen in apple vinegar. At present, women are at increased risk of metabolic syndrome after menopause (Hildrum et al., 2007; Ruan et al., 2010). In menopausal women, hormonal imbalance leads to increased rates of obesity and risk of cardiovascular disease due to increased internal fat and decreased functioning of vascular protection functions (Franzosi, 2006; Broussard and Magnus, 2008). Obesity caused by these factors may increase the risk of insulin resistance and cause diabetes (Lee et al., 2000). At the same time,

Received 7 May 2019; Accepted 17 January 2020; Published online 31 March 2020

Correspondence to Mi Young Kang, Tel: +82-53-950-6235, E-mail: mykang@knu.ac.kr
Author information: Su Jin Nam (Graduate Student), Soo Im Chung (Professor), Mi Young Kang (Professor)

functional disorders involving an imbalance of insulin and glucagon levels cause serious complications, such as loss of carbohydrate, protein, and lipid metabolic control (Chung et al., 2016). As a consequence, we considered ways to curb the rate of diabetes development resulting from obesity caused by menopause.

In this study we explored the change in amino acid content occurring after deposition of acorn pollen in apple vinegar. Then, using pollen extract supernatants and precipitations, analyzed biomarkers related to glucose metabolism.

MATERIALS AND METHODS

Materials

Acorn pollen was provided and supplied by the beekeeping farming association of incorporation (Yeongju, Korea) and was stored frozen until analysis. Apple vinegar (Sempio, Yeongcheon, Korea) used for extraction was purchased by E-mart (Daegu, Korea).

Acorn pollen was added to 5 times apple vinegar and leached by stirring at 40°C for 30 days. The separated supernatant was used to determine the amino acid content and enzyme inhibitory activity, and the precipitate was used for surface analysis using an electron microscope. Supernatants and precipitates that had been deposited for 30 days were used in animal experiments.

Amino acid component analysis

The pollen supernatant was mixed with apple vinegar, then collected and filtered through a 0.45 μm syringe (Advantec Toyo Kaisha, Ltd., Tokyo, Japan). Amino acid component analysis was carried out using an automated amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). Then, 20 μL of samples were injected into an ion exchange column. The temperature of the column was set at 50°C and the temperature of the reactor was set at 135°C.

Observation of the surface of the acidic-treated acorn pollen

A scanning electron microscope (SEM, SU8220, Hitachi) was used to observe changes in the sedimentary pollen surface over the period of time the pollen was stirred in the apple vinegar.

Inhibition assays of diabetic-related enzymes

The inhibitory activity of α-amylase was measured using the method of Kazeem et al. (2013). The sample extract (125 μL) was added to α-amylase (62.5 μL) and 200 mM potassium phosphate buffer (pH 6.8, 62.5 μL) and incubated at 37°C for 10 min. Then, 1% starch (125 μL) was added to the mixture and the mixture was incubated for another 5 min. To produce a colored solution, 48 mM 3,5-dinitrosalicylic acid (125 μL) was added. The colored solution was then incubated at 100°C for 15 min and cooled on ice. Finally, distilled water (1.5 mL) was added to the solution and the absorbance was measured at 405 nm.

The inhibitory activity of α-glucosidase was measured using the method of Hong et al. (2013a). Samples (50 μL) were added to α-glucosidase (100 μL) and reaction incubated in a water bath (37°C, 10 min). Then, 1 mM *p*-nitrophenyl-α-D-glucopyranoside (in potassuim phosphate buffer, pH 6.9) was added. After reacting for 20 min in the water bath at 37°C, 1 M Na_2CO_3 (1 mL) was added to stop the reaction, and the absorbance was measured at 405 nm.

Xanthine oxidase inhibition was measured according to the method described in by Stirpe and Della Corte (1969). Dissolved 2 mM xanthine (0.2 mL) was added samples (100 μL) and 0.1 M potassium phosphate buffer (pH 7.5, 600 μL) and reactions were incubated in a water bath (37°C, 5 min). Then, 1 N HCl (1 mL) was added stop the reaction, and the absorbance was measured at 292 nm.

The inhibitory activity of lipase was measured by modifying the methods of Lee et al. (2010). To create the substrate solution, 10 mM 3-(N-morpholino) propabelsulfonic acid, 4-morpholinepropanesulfonic acid, and Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 6.8) were mixed. The substrate solution (860 μL) was added to 100 μL of sample and reactions incubated for 5 min in a water bath at 37°C. Then, 10 mM *p*-nitrophenyl butyrate (40 μL) was added to mixture and reactions were incubated for 15 min before the absorbance was measured at 400 nm.

Animals and diet

The animal lab was maintained at 25±2°C and 50% humidity, with regular lighting from 6 am to 6 pm. Experimental animals were 12 weeks old Sprague-Dawley rats. Sixteen normal rats and 32 rats that were ovariectomized were obtained from Central Lab. Animal Inc. (Seoul, Korea). Rats were first acclimatized to their new environment for 1 week. After one week, two experiments were carried out confirm the effects of ingestion of deposited pollen supernatants and precipitates on glucose metabolism of ovariectomized rats. In the first experiment (Sup-O), supernatants were orally administered to ovariectomized rats. In the second experiment (Ppt-F), precipitates were mixed with feed before being administered to rats. The sham rats were randomly assigned to the normal control of each the Sup-O and Ppt-F experiments. Ovariectomized rats were randomly assigned by dividing into control and experimental group for the Sup-O and Ppt-F experiments. Sup-O experiments: (1) SHAM-O, sham-operated rats received oral administration of distilled water (7 mL/kg/d); (2) OVX-OC, ovariectomized

rats received oral administration of distilled water (7 mL/kg/d); (3) OVX-OP, ovariectomized rats received oral administration of supernatants apple vinegar-treated pollen (7 mL/kg/d) (Setorki et al., 2010). Ppt-F experiments: (1) SHAM-F, sham-operated rats received AIN93M; (2) OVX-FC, ovariectomized rats received AIN93M; (3) OVX-FP, ovariectomized rats received AIN93M with 10% precipitates from apple vinegar-treated pollen (Salles et al., 2014). Oral administration of Sup-O was provided using a feeding needle each morning for 4 weeks, and the AIN-93M diet was provided in feed. Ppt-F experiments were conducted for 12 weeks. The feed was purchased from FeedLab (Guri, Korea), and the components of diet are shown in Table 1. During the experiment, the feed and drinking water were freely consumed. At the end of the experimental period, animals were sacrificed and organ tissues and blood were collected, weighed, and stored in a freezer at −70°C. Blood was collected via the arteries of rat using ethylenediaminetetraacetic acid (EDTA), and centrifuged at 1,000 *g* (4°C, 15 min). Finally, the upper layer obtained was stored at −70°C for use in experiments.

The study was approved by the Animal Experiment Ethics Committee at Kyungpook National University (KUN 2016-0117).

Collection of animal cell tissues and microsomal factions as enzymatic fractions

Enzymes were isolated from hepatic and nephritic tissues. Buffer (pH 7.4, 5 mL) containing 0.1 mM triethanolamine, 20 mM EDTA, and 2 mM dithiothreitol was mixed with cell tissues (0.3 g). After homogenizing, mixtures were centrifuged at 1,000 *g* (4°C, 15 min). After the upper layers (3.5 mL) were removed, the mixtures were further centrifuged at 10,000 *g* (4°C, 20 min). Buffer solution (3 mL) was then added to the precipitated residues, then the mixtures were centrifuged at 10,000 *g* (4°C, 20 min) to obtain mitochondrial fractions. The upper layer (cytosol fraction) was then separated by ultra-centrifugation at 105,000 *g* (4°C, 1 h). Buffer solution (3 mL) was then added to residual fractions and the supernatants were removed by ultra-centrifugation at 105,000 *g* (4°C, 1 h). Residues of microsomal fractions were used in experiments.

Table 1. Components of the experimental diets (unit: g)

Ingredients	AIN-93M[1)]	Pollen feed
Casein	140.00	123.89
Sucrose	100.00	100.00
Dextrose	155.00	155.00
Corn starch	465.70	384.88
Cellulose	50.00	50.00
Soybean oil	40.00	36.93
Mineral mix	35.00	35.00
Vitamin mix	10.00	10.00
L-Cystine	1.80	1.80
Choline bitartrate	2.50	2.50
Pollen vinegar precipitated[2)]		100.00
Total	1,000.0	1,000.0

[1)]Provided to all groups except for OVX-FP of Ppt-F.
[2)]Provided to OVX-FP of Ppt-F groups.

Blood glucose and insulin density measurements

The change in blood glucose concentration from pre-intake to post-intake of pollen was determined according to the experimental diets. Blood glucose was measured using a glucose meter (Accu-Chek, Roche Diagnostics, Basel, Switzerland) in blood from the rats' tail veins after a 12-h feed. Plasma insulin concentrations were measured using the rat insulin enzyme-linked immunosorbent assay kit (Mybiosource, San Diego, CA, USA).

Biomarker measurement of glucose metabolism in cellular tissue

The activity of glucose-6-phosphatase (G6pase) was measured by the method described by Alegre et al. (1988) with modifications. A buffer solution was formed through mixing 100 mM EDTA and 26.5 mM glucose-6-phosphate. Next 0.2 M nicotinamide adenine dinucleotide phosphate (965 μL), mutarotase (aldose-1-epimerase) and glucose dehydrogenase (10 μL) were added and the mixture was incubated for 4 min in a water bath at 37°C. The change in absorbance was measured at 340 nm. Activation of G6pase was expressed in nmol nicotinamide adenine dinucleotide phosphate hydrogen oxidized/min mg protein.

Glucokinase (GK) activity was measured by following the method by Davidson and Arion (1987) with modifications. A buffer solution was formed through mixing 50 mM Hepes (pH 7.4), 100 mM KCl, 2.5 mM dithioerythritol, 7.5 mM $MgCl_2$, and 10 mM glucose. Then 4 units glucose-6-phosphate dehydrogenase (10 μL), 500 mM adenosine triphosphate (10 μL), and a cytosol fraction (10 μL) were added to the buffer solution (910 μL) and reactions were incubated in a water bath at 37°C. The change in absorbance was measured at 340 nm. GK activity was expressed in nmol of nicotinamide adenine dinucleotide hydrogen (NADH) generated per minute per mg of cytosolic protein (nmol NADH oxidized/min/mg protein).

Statistical analysis

All data are presented as mean±standard error of the mean. Data were evaluated by one-way ANOVA using Statistical Package for Social Sciences software program version 22 (SPSS Inc., Chicago, IL, USA) and the differences between means were assessed by Tukey's test. Statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Physico-chemical properties of acidic-treated acorn pollen

The physiological characteristics of acid-treated acorn pollen were analyzed by the distribution of amino acid amongst the components extracted through pollen holes.

Table 2. Types and variations in the content of amino acids released from pollen holes by acidic-treated precipitates (unit: μg/mL)

	Control	30 days
Amino acids		
Histidine	36.65	80.64
Isoleucine	102.54	216.15
Leucine	275.56	540.35
Lysine	34.93	43.71
Phenylalanine	189.61	398.31
Threonine	113.47	211.55
Valine	103.89	288.60
Methionine	106.39	175.76
Alanine	207.60	331.09
Arginine	1.32	1.62
Aspartic acid	169.33	454.94
Cystine	72.64	73.95
Glutamic acid	473.72	722.43
Glycine	58.77	118.40
Proline	163.56	259.46
Serine	199.65	267.86
Tyrosine	107.06	407.73
Nnitrogen compounds in the form of functional amino acids		
Taurine	ND	12.24
γ-Aminobutyric acid	126.15	158.96
β-Amino isobutyric acid	110.54	112.49
α-Amino-*n*-butyric acid	19.93	33.60
β-Alanine	54.12	69.38
α-Amino adipic acid	51.67	168.70
Anserine	4.28	11.99
Carnosine	25.68	83.58
Citrulline	ND	38.59
Cystathionine	ND	31.93
Ethanol amine	109.81	117.93
1-Methylhistidine	297.98	459.32
3-Methylhistidine	67.31	196.21
Phosphoserine	35.71	46.63
Sarcosine	95.23	161.37
Total	3,415.10	6,295.47

ND, not detected.

SEM was used to observe changes to the surface of the pollen grains by acid treatment. The amino acids in the materials believed to have been released through the pollen holes during the 30-day process are presented in Table 2. These data were calculated by subtracting the amino acids contained in the apple vinegar from the total amino acid content. Seventeen of the 20 amino acids needed for human health were extracted from the pollen, including seven of the eight essential amino acids necessary for human growth and maintenance of health. In addition, up to 15 of so-called 'functional' amino acids containing nitrogen were found. The most abundant amino acid was glutamic acid, a high about of which has previous been reported in pollen (Lee et al., 1997; Hong et al., 2013a). In this study, glutamic acid was the most abundant amino acid regardless of the acid treatment. As shown in Table 2, nitrogen compounds in the form of functional amino acids were also extracted from pollen. SEM scans of the precipitates were carried out to assess the degree of surface damage following acid treatment. Although surface corrosion and cracking of the pollen were expected following prolonged treatment with apple vinegar, there were no surface corrosion or cracks observed. Since nutrients were extracted from the pollen, it can be concluded that they eluted through tiny holes on the surface of the acorn pollen (Hong et al., 2013b).

Analysis of the inhibitory effect of acidic-treated acorn pollen on diabetes-related enzymes

The inhibitory activity of acid-treated acorn pollen on diabetes related hydrolase, α-glucosidase, xanthine oxidase, and lipase is shown in Table 3. The control group (apple vinegar) also showed inhibitory effects on the activity of these diabetes-related enzymes. Prolonged treatment acorn pollen with acid significantly increased the inhibitory activity. Compared with day 1, inhibition of xanthine oxidase was doubled after three days and increased by 80-fold after 30 days, and inhibition of α-amylase was doubled after three days and increased 40 fold after 30 days. However, there was little difference in the enzymatic activity of α-glucosidase and lipase after 30 days. According to Bhandari et al. (2008), medicinal plants with anti-diabetic properties have inhibitory actions, such as

Table 3. Inhibitory activity of acidic-treated acorn pollen on diabetic-related enzymes associated (unit: nmol/min/mL)

Enzymes	Apple vinegar	Acorn pollen deposited with apple vinegar	
		for 3 day	for 30 days
α-Amylase	87.96±1.10^{a}	167.94±6.28^{b}	2,397.41±23.89^{c}
α-Glucosidase	1.03±0.06^{a}	1.64±0.04^{b}	3.64±0.03^{c}
Xanthine oxidase	27.49±0.26^{a}	77.31±1.58^{b}	2,283.53±14.61^{c}
Lipase	5.25±0.05^{a}	8.08±0.02^{b}	9.01±0.06^{c}

Mean±standard error of the mean (n=3).
Means of the same row without corresponding letters (a-c) are significantly different by Tukey's test (P<0.05).

Table 4. Body weight gain, dietary intake, liver and kidney weight and fat contents of ovariectomized rats fed with the acid-treated acorn pollen

		Weight gain (g/d)	Feed intake (g/d)	Liver (g)	Kidney (g)	Fat content (g)
Sup-O	SHAM-O	1.43±0.01[a]	22.43±0.33[a]	10.91±0.59[ns]	1.94±0.14[ns]	20.02±0.20[a]
	OVX-OC	2.26±0.03[c]	27.50±0.22[c]	10.24±0.52	2.15±0.06	25.63±1.10[b]
	OVX-OS	1.85±0.01[b]	23.86±0.07[b]	8.73±0.37	1.94±0.04	21.71±0.50[a]
Ppt-F	SHAM-F	1.35±0.03[e]	22.09±0.10[e]	9.47±0.32[ef]	2.13±0.07[ns]	24.10±0.30[e]
	OVX-FC	2.72±0.01[g]	30.05±0.36[g]	10.84±0.51[f]	1.98±0.06	28.75±0.60[f]
	OVX-FP	1.64±0.02[f]	25.42±0.10[f]	8.28±0.33[e]	2.02±0.06	28.70±0.45[f]

Values are mean±standard error of the mean (n=8).
Different letters indicate a significant difference (P<0.05) in the same column in Sup-O (a-c) and Ppt-F (e-g).
[ns]Not significant.
Sup-O, oral administration of supernatants of acid-treated acorn pollen; Ppt-F, feed by a mixture of precipitates of acid-treated acorn pollen; SHAM-O, sham treated rats receiving oral administration of distilled water (7 mL/kg/d); OVX-OC, ovariectomized rats receiving oral administration of distilled water (7 mL/kg/d); OVX-OS, ovariectomized rats receiving oral administration of supernatant from pollen treated with apple vinegar (7 mL/kg/d); SHAM-F, sham operated rats receiving AIN93M; OVX-FC: ovariectomized rats receiving AIN93M; OVX-FP, ovariectomized rats receiving AIN93M with 10% precipitate from pollen treated with apple vinegar.

α-amylase and α-glucosidase, therefore pollen may have effect blood glucose degradation through the inhibiting these enzymes.

Changes in weight gain, dietary intake, liver and kidney weight, and fat content of kidney ambient and abdominal white fat

Rats that were ovariectomized to induce menopause gained weight during the experiment. The weights of the liver and kidneys and the total fat content were measured separately. The total fat content was determined using white fat removed from around the kidney and the abdomen. The increase in weight gain and food intake was higher in rats receiving acid-treated supernatants (Sup-O) and precipitants (Ppt-F) compared with those in the SHAM-O and SHAM-F groups (Table 4). These results are consistent with results reported by Kim et al. (2006), which showed that appetite and fat content increases after menopause, which may lead to obesity.

Rats receiving both the supernatant (OVX-OS) or precipitant (OVX-FP) in addition to acid-treated pollen showed significant decreases in weight and food intake. However, there was no significant difference in weight of the liver or kidneys between rats in the SHAM group, ovariectomized control groups (OVX-OC and OVX-FC) and the experimental groups (OVX-OS and OVX-FP). However, the decreases in total fat content (including significant decreases in kidney ambient and abdominal white fat) following administration with acid-treated supernatants (OVX-OS) are encouraging.

Effects on blood glucose levels

The change in blood glucose level following treatment of ovariectomized rats with acid-treated Sup-O or Ppt-F are shown in Fig. 1. Blood glucose levels of rats in the OVX-OC group was increased by menopause. However, in treatment with Sup-O, decreased blood glucose levels. These same results were obtained following Ppt-F. Similarly, Lee et al. (1994) reported that ingestion of pine pollen reduces blood glucose in rats receiving a high fat diet. These results suggest that acid-treated pollen may be used as a functional food for blood glucose control.

Analysis of biomarkers for glycolysis

Blood insulin concentration is a biomarker for glycemic metabolism. Insulin is released from pancreatic β-cells maintain blood glucose levels at a constant level. As levels of blood glucose increase, insulin is released from the pancreas and is taken up by cells, where it is converted into fatty acids or induces glucose oxidation. Menopause increases blood insulin levels, which usually increases appetite and fat accumulation, thus increasing the risk of insulin-resistant diabetes (Dubuc, 1985; Kahn and Flier, 2000). As shown in Table 5, the ovariectomized rats (OVX-OC and OVX-FC) may have significantly higher blood insulin concentrations than rats in the SHAM-O and SHAM-F groups.

We measured the activity of G6pase and GK from tissue of liver and kidney as biomarkers of glycemic metabolism. G6pase is an enzyme involved in gluconeogenesis, whereby it promotes production of glucose and to increase blood glucose levels (Lee et al., 2012). GK, another biomarker for glycemic metabolism, is an enzyme involved in the first stage of glycolysis which promotes conversion of glucose into glycogen. Glycogen can be biologically stored, thus reducing the risk of diabetes (Iynedjian et al., 1988; Postic et al., 1999; She et al., 2000; van Schaftingen and Gerin, 2002). We therefore used G6pase and GK as biomarkers to test the effect of acid-treated pollen on glycemic metabolism in post-menopausal.

Oral administration of Sup-O significantly increased the activity of G6pase, in the liver and kidneys of postmenopausal ovariectomized rats (OVX-OC), resulting in an increase in blood glucose. However, the activity of G6pase was decreased in both liver and kidney tissues supplementation with OVX-OS. Similar results were obtained

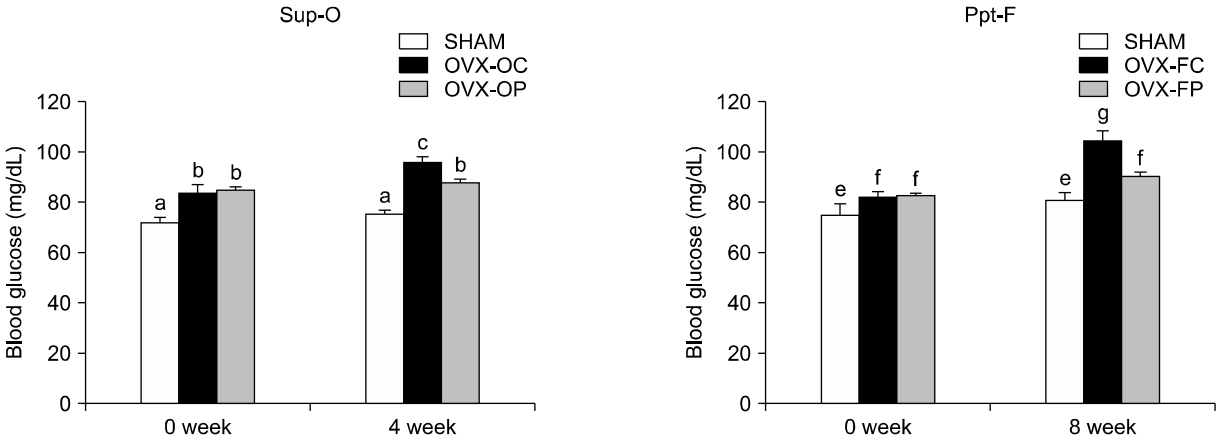

Fig. 1. Blood glucose analysis of ovariectomized rats fed with the acidic-treated acorn pollen. Values are mean±standard error of the mean (n=8). Different letters indicate a significant difference (P<0.05) same time in Sup-O (a-c) and Ppt-F (e-g). Sup-O, oral administration of supernatant of the acidic-treated acorn pollen; Ppt-F, feed by mixture of precipitate of the acidic-treated acorn pollen; SHAM-O, sham-operated rats receiving oral administration of distilled water (7 mL/kg/d); OVX-OC, ovariectomized rats receiving oral administration of distilled water (7 mL/kg/d); OVX-OS, ovariectomized rats receiving oral administration of supernatant from pollen on the apple vinegar (7 mL/kg/d); SHAM-F, sham-operated rats feeding AIN93M; OVX-FC, ovariectomized rats feeding AIN93M; OVX-FP, ovariectomized rats feeding AIN93M with 10% precipitate from pollen on the apple vinegar.

Table 5. Insulin and glucose regulating enzyme activity of ovariectomized rats fed acid-treated acorn pollen

		Plasma (ng/mL)	Hepatic tissue (nmol/min/mg protein)		Nephritic tissue (nmol/min/mg protein)	
		Insulin	G6pase	GK	G6pase	GK
Sup-O	SHAM-O	1.08±0.04^{a}	12.80±0.34^{a}	6.61±0.20^{c}	28.07±2.27^{a}	8.05±0.54^{b}
	OVX-OC	1.38±0.03^{c}	54.91±1.32^{c}	0.90±0.01^{a}	51.42±2.53^{c}	2.89±0.65^{a}
	OVX-OS	1.20±0.04^{b}	47.86±1.90^{b}	1.65±0.15^{b}	39.12±2.51^{b}	5.80±0.74^{b}
Ppt-F	SHAM-F	1.03±0.04^{e}	21.25±0.56^{e}	6.59±0.57^{g}	31.66±1.69^{e}	5.51±0.42^{f}
	OVX-FC	1.31±0.03^{g}	50.86±2.14^{g}	1.62±0.23^{e}	51.76±2.37^{g}	2.77±0.27^{e}
	OVX-FP	1.10±0.05^{f}	36.27±1.72^{f}	2.57±0.05^{f}	42.58±2.63^{f}	3.86±0.28^{e}

Values are mean±standard error of the mean (n=8).
Different letters indicate a significant difference (P<0.05) in the same column in Sup-O (a-c) and Ppt-F (e-g).
Sup-O, oral administration of supernatant of acid-treated acorn pollen; Ppt-F, feed by mixture of precipitates of acid-treated acorn pollen; SHAM-O, sham treated rats receiving oral administration of distilled water (7 mL/kg/d); OVX-OC, ovariectomized rats receiving oral administration of distilled water (7 mL/kg/d); OVX-OS, ovariectomized rats receiving oral administration of supernatant from pollen treated with apple vinegar (7 mL/kg/d); SHAM-F, sham treated rats feeding AIN93M; OVX-FC: ovariectomized rats receiving AIN93M; OVX-FP, ovariectomized rats receiving AIN93M with 10% precipitates of pollen treated with apple vinegar; GK, glucokinase; G6pase, glucose-6-phosphatase.

by supplementation with Ppt-F. In postmenopausal OVX-FC, G6pase was significantly increased in both liver and kidney tissues. However, the activity of G6pase in hepatic and nephritic tissues was decreased by supplementation with OVX-FP. The activity of GK, which is involved in promoting blood storage of glycogen and is therefore one factor that can control the risk of diabetes, was significantly decreased in postmenopausal OVX-OC and OVX-FC. Although the extent of the increases differed, the activity of GK both in liver and kidney tissues was increased by the supplementation with OVX-OS and OVX-FP. Pari and Amarnath Satheesh (2004) showed that G6pase inhibitors lower blood glucose levels in experimental animal models of diabetes. Further, Kim et al. (2004) reported that in diabetes GK activity is reduced and glucose synthesized in the liver is released into the blood, which disrupts physiological control of blood glucose levels. Therefore, the results of our present study suggest that pollen helps recover the activities of enzymes involves in regulating blood glucose levels, which are decreased by menopause and obesity.

In conclusion, we carried out a series of studies to review acid-treated pollen obtained by deposition of acorn pollens in apple vinegar as a functional food. As commercial apple vinegar was used for acid treatment, consuming this by proper dilution is possible. We treated pollen with acid for 30 days since preliminary results showed that after 30 days there are no increases in extraction of functional component that may improve health. After 30 days of processing, the apple vinegar had a pH 2.56, which did not induce changes to or crack to the surface of the pollen. It may be inferred that substances inside the pollen are extracted through the holes in the surface, such as seven of the eight essential amino acids

necessary for growth and maintenance of human health. In addition, there up to 15 types of 'functional' amino acids which contain nitrogen compounds. Acid-treated pollen solutions containing high-quality nitrogen compounds are believed to be effective for controlling obesity, such as through inhibiting the activity of carbohydrates and fat digestive enzymes. Thus, the effect on carbohydrate and fat hydrolysis can be expected to induce anti-obesity effects and improve glucose metabolism after menopause. We also investigated this hypothesis through analyzing biomarkers associated with glycolysis and obesity control in menopausal ovariectomized rats. Treatment with acid treatment pollen significantly decreased the activity of the G6pase, an enzyme involved in gluconeogenesis, which is activated after menopause to increase serum glucose levels, often leading to obesity. Furthermore, GK, an enzyme that involved in the first stage of glycolysis, often shows decreased activity following menopause, which decreases blood glucose levels. We showed that acid-treated pollen was effective in restoring GK activity. These results that acid-treated pollen may be used as a functional health food material for post-menopausal metabolism.

ACKNOWLEDGEMENTS

This research was supported by the Cooperative Research Program for Agriculture Science and Technology Development project (No. PJ013140), Rural Development Administration, Republic of Korea.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

Abouda Z, Zerdani I, Kalalou I, Faid M, Ahami MT. The antibacterial activity of Moroccan bee bread and bee-pollen (fresh and dried) against pathogenic bacteria. Res J Microbiol. 2011. 6: 376-384.

Alegre M, Ciudad CJ, Fillat C, Guinovart JJ. Determination of glucose-6-phosphatase activity using the glucose dehydrogenase-coupled reaction. Anal Biochem. 1988. 173:185-189.

Andradea P, Ferreres F, Gil MI, Tomás-Barberán FA. Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. Food Chem. 1997. 60: 79-84.

Bhandari MR, Jong-Anurakkun N, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). Food Chem. 2008. 106:247-252.

Blackmore S, Wortley AH, Skvarla JJ, Gabarayeva NI, Rowley JR. Developmental origins of structural diversity in pollen walls of Compositae. Plant Syst Evol. 2010. 284:17-32.

Broussard DL, Magnus JH. Coronary heart disease risk and bone mineral density among U.S. women and men. J Womens Health. 2008. 17:479-490.

Choi SJ, Jeong Y. Effect of proteases on the extraction of crude protein and reducing sugar in pollen. J Korean Soc Food Sci Nutr. 2004. 33:1353-1358.

Chung SI, Kang MY, Lee SC. Inhibitory effect of germinated pigmented rice on key metabolic enzymes associated with diabetes and hyperglycemia. Int J Food Nutr Sci. 2016. 5:33-40.

Chung YG, Yoon SH, Kwon JS, Bae MJ. Nutritional and biochemical studies on the pollen loads-1. Studies on lipid compositions of sunflower pollen load and effects of its pollen load on liver cholesterol metabolism in mouse. J Korean Soc Food Nutr. 1984. 13:169-174.

Davidson AL, Arion WJ. Factors underlying significant underestimations of glucokinase activity in crude liver extracts: physiological implications of higher cellular activity. Arch Biochem Biophys. 1987. 253:156-167.

Dubuc PU. Effects of estrogen on food intake, body weight, and temperature of male and female obese mice. Proc Soc Exp Biol Med. 1985. 180:468-473.

Fang K, Wang Y, Yu T, Zhang L, Baluška F, Šamaj J, et al. Isolation of de-exined pollen and cytological studies of the pollen intines of *Pinus bungeana* Zucc. Ex Endl. and *Picea wilsonii* Mast. Flora. 2008. 203:332-340.

Franzosi MG. Should we continue to use BMI as a cardiovascular risk factor?. Lancet. 2006. 368:624-625.

Hildrum B, Mykletun A, Hole T, Midthjell K, Dahl AA. Age-specific prevalence of the metabolic syndrome defined by the International Diabetes Federation and the National Cholesterol Education Program: the Norwegian HUNT 2 study. BMC Public Health. 2007. 7:220. https://doi.org/10.1186/1471-2458-7-220

Hong HC, Li SL, Zhang XQ, Ye WC, Zhang QW. Flavonoids with α-glucosidase inhibitory activities and their contents in the leaves of *Morus atropurpurea*. Chin Med. 2013a. 8:19. https://doi.org/10.1186/1749-8546-8-19

Hong IP, Lee MY, Woo SO, Sim HS, Choi YS, Han SM, et al. Change of chemical composition in acorn pollen after physical treatment. Korean J Apiculture. 2013b. 28:217-221.

Hong IP, Woo SO, Han SM, Yeo JH, Cho ML, Ju WT, et al. Morphology and antioxidant activity in pollens of Korean oak and darea (*Actinidia arguta*). J Apic. 2014. 29:137-142.

Iynedjian PB, Gjinovci A, Renold AE. Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. J Biol Chem. 1988. 263:740-744.

Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000. 106:473-481.

Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. Biomed Res Int. 2013. 2013:527570. https://doi.org/10.1155/2013/527570

Kim DS. Effect of larva gut enzyme on pollen. Korean J Food Sci Technol. 1989. 21:404-408.

Kim JG, Son JH. Progress of chemical composition on pulverization of pollen loads. Korean J Apiculture. 1990. 5:23-30.

Kim JH, Lee KH, Kim ES, Park SB. A study on components of pollen load. J Korean Soc Food Sci Nutr. 1992. 21:566-572.

Kim JW, Shin SC, Kim BK. Studies on pollen preparations and as a health food (I). Kor J Pharmacogn. 1984. 15:147-149.

Kim SK, Sunwoo JG, Lee EJ. Relation of mineral nutrition status and climacteric symptoms in pre- and postmenopausal women. Korean J Nutr. 2006. 39:121-132.

Kim SY, Kim HI, Kim TH, Im SS, Park SK, Lee IK, et al. SREBP-1c mediates the insulin-dependent hepatic glucokinase expression. J Biol Chem. 2004. 279:30823-30829.

Kress WJ, Stone DE, Sellers SC. Ultrastructure of exine-less pol-

len: *Heliconia* (Heliconiaceae). Am J Bot. 1978. 65:1064-1076.
Kroyer G, Hegedus N. Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innov Food Sci Emerg Technol. 2001. 2:171-174.
Lee BY, Choi HD, Hwang JB. Components analysis of Korean pollens and pollen extracts. Korean J Food Sci Technol. 1997. 29:869-875.
Lee EM, Lee SS, Chung BY, Cho JY, Lee IC, Ahn SR, et al. Pancreatic lipase inhibition by C-glycosidic flavones isolated from *Eremochloa ophiuroides*. Molecules. 2010. 15:8251-8259.
Lee J, Seo KI, Kim MJ, Lee SJ, Park EM, Lee MK. Chlorogenic acid enhances glucose metabolism and antioxidant system in high-fat diet and streptozotocin-induced diabetic mice. J Korean Soc Food Sci Nutr. 2012. 41:774-781.
Lee JH, Chung YK, Park HM, Park JS, Yumiko H, Yeoum SG. A study on climacteric symptoms in Korean midlife women. J Korean Soc Menopause. 2000. 6:141-156.
Lee ST. Palynology and plant systematics. Korean J Apiculture. 1986. 1:46-53.
Lee YJ, Park MH, Hwang SW, Bae MJ, Han JP. Effect of pine pollen on serum and liver lipids in rats on a fed high fat diet. J Korean Soc Food Nutr. 1994. 23:192-197.
Li F, Yuan QP, Rashid F. Isolation, purification and immunobiological activity of a new water-soluble bee pollen polysaccharide from *Crataegus pinnatifida Bge*. Carbohydr Polym. 2009. 78: 80-88.
Llnskens HF, Jorde W. Pollen as food and medicine—a review. Econ Bot. 1997. 51:78. https://doi.org/10.1007/BF02910407
Pari L, Amarnath Satheesh M. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol. 2004. 91:109-113.
Postic C, Shiota M, Niswender KD, Jetton TL, Chen Y, Moates JM, et al. Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic β cell-specific gene knockouts using Cre recombinase. J Biol Chem. 1999. 274:305-315.
Roulston TH, Cane JH. Pollen nutritional content and digestibility for animals. Plant Syst Evol. 2000. 222:187-209.
Ruan X, Jin J, Hua L, Liu Y, Wang J, Liu S. The prevalence of metabolic syndrome in Chinese postmenopausal women and the optimum body composition indices to predict it. Menopause. 2010. 17:566-570.
Salles J, Cardinault N, Patrac V, Berry A, Giraudet C, Collin ML, et al. Bee pollen improves muscle protein and energy metabolism in malnourished old rats through interfering with the mTOR signaling pathway and mitochondrial activity. Nutrients. 2014. 6:5500-5516.
Setorki M, Asgary S, Eidi A, Rohani AH, Khazaei M. Acute effects of vinegar intake on some biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. Lipids Health Dis. 2010. 9:10. https://doi.org/10.1186/1476-511X-9-10
She P, Shiota M, Shelton KD, Chalkley R, Postic C, Magnuson MA. Phosphoenolpyruvate carboxykinase is necessary for the integration of hepatic energy metabolism. Mol Cell Biol. 2000. 20:6508-6517.
Stirpe F, Della Corte E. The regulation of rat liver xanthine oxidase. Conversion *in vitro* of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J Biol Chem. 1969. 244: 3855-3863.
Suk KD, Kim MK. Gross chemical analysis for honey and pollen load. Kor J Pharmacog. 1983. 14:197-200.
van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochem J. 2002. 362:513-532.