

氏 名	ツウ ショウコウ 慈 照紅
本籍（国籍）	中華人民共和国
学 位 の 種 類	博士（農学）
学 位 記 番 号	連研第 734 号
学位授与年月日	平成 3 1 年 3 月 2 2 日
学位授与の要件	学位規則第 5 条第 1 項該当課程博士
研究科及び専攻	連合農学研究科 生物資源科学
学位論文題目	<b>The functional components and functionalities of scarlet runner beans（花豆の機能性成分と機能性に関する研究）</b>
学位審査委員	主査 帯広畜産大学教授 小嶋 道之 副査 村山 哲也(山形 教授),板谷 篤司(帯広 准教授),塚本 知玄 (岩手 教授)

## 論 文 の 内 容 の 要 旨

Scarlet runner beans are a valuable source of many nutrients, including proteins, starch, dietary fiber, and oligosaccharides, and are used in various foods in Japan. To extend our knowledge of the effects of scarlet runner beans on human health, we analyzed the color, polyphenol and procyanidin content, DPPH radical scavenging activity, reducing power, and  $\alpha$ -glucosidase inhibitory activity of various scarlet runner beans. The  $L^*$  and  $C$  values were the highest for scarlet runner beans (white) and lowest for scarlet runner beans (black). Scarlet runner beans (purple) and scarlet runner beans (brown) showed significant higher polyphenol and procyanidin content than those of scarlet runner beans (white) and scarlet runner beans (mixed).

Scarlet runner beans (brown) and scarlet runner beans (mixed) showed the highest DPPH radical scavenging activity and reducing power. Scarlet runner beans (white) had the lowest ratio of oligomeric and polymeric polyphenols and the lowest DPPH radical scavenging activity and reducing power. We found a positive correlation between polyphenol content and both DPPH radical scavenging activity and reducing power. Moreover, polyphenols from various scarlet runner beans inhibited the activity of  $\alpha$ -glucosidase in a dose-dependent manner. The polyphenols (50  $\mu\text{g/mL}$ ) of scarlet runner beans (black)

showed the highest  $\alpha$ -glucosidase inhibitory activity (85.7%), and those of scarlet runner beans (white) showed the lowest inhibitory activity (53.8%). Scarlet runner beans (black) had a lower  $IC_{50}$  value (26.4  $\mu$ g/mL) and scarlet runner beans (white) had a higher  $IC_{50}$  value (58.4  $\mu$ g/mL) than those of other scarlet runner beans.

Proanthocyanidins are oligomeric or polymeric flavonoids found in several plants. Polyphenol content was 188.72 mg/g seed coat of scarlet runner beans (purple), and those of proanthocyanidin content was 179.51 mg/g seed coat. The proportion of proanthocyanidins were 95% in polyphenols of scarlet runner beans (purple) seed coat. The seed coat of scarlet runner beans (purple) contained mainly oligomeric and polymeric polyphenols, and their proportions were 47% and 53%, respectively. Moreover, polymeric polyphenols exhibited higher DPPH radical scavenging activity than that of oligomeric polyphenols. Oligomeric and polymeric polyphenols demonstrated a significant ( $p < 0.05$ ) dose-dependent suppressive effect on lipase, with  $IC_{50}$  values of  $3.88 \pm 0.35$ , and  $1.84 \pm 0.46$   $\mu$ g/mL, respectively. These results indicates that more highly polymerized polyphenols exhibited more potent DPPH radical scavenging activity and lipase inhibitory activity.

To investigate the composition and calculate the mDP values for oligomeric and polymeric polyphenols, thiolysis was carried out and the reaction products were analyzed by RP-HPLC. We detected (+)-catechin and (–)-epicatechin monomers in reaction products of oligomeric and polymeric polyphenols fractions, indicating that these constituted the terminal units of the corresponding proanthocyanidins. Moreover, the (–)-epicatechin derivative was the compound with the largest peak area, suggesting that (–)-epicatechin was the main constituent compound for proanthocyanidins in both fractions. Regarding mDP, polymeric polyphenols had a higher value (9.6) than oligomeric polyphenols (3.8). In both fractions, polyphenols were of the B-type configuration.

Moreover, we examined polyphenols from seed coat of scarlet runner beans (purple) (SRPA) effect on mice fed a high-fat diet. Male mice were assigned to the following

seven-subject dietary groups: (1) high-fat diet (HF); (2) high-fat diet supplemented with 0.5% SRPA (0.5% SRPAHF); (3) high-fat diet supplemented with 1.0% SRPA (1.0% SRPAHF), and; (4) standard chow (SW) for 15 weeks. SRPA supplementation decreased body weight gain; liver and kidney weight; liver, perirenal, peritesticular, and periintestinal fat content; liver cholesterol level, and; serum neutral lipid and cholesterol levels, whereas the fecal lipid content and the liver anti-oxidative capacity were increased. These findings suggest a potential use of SRPAs as a dietary supplement exerting anti-obesity effects through the inhibition of fat digestive enzymes.

Moreover, we examined the inhibitory activities of SRPAs on  $\alpha$ -amylase and  $\alpha$ -glucosidase. SRPAs demonstrated dose-dependent suppressive effect  $\alpha$ -amylase and  $\alpha$ -glucosidase, with  $IC_{50}$  values of  $15.08 \pm 0.24$ , and  $11.89 \pm 0.28$   $\mu\text{g/mL}$ , respectively. SRPAs were tested for their inhibitory effects on the elevation of blood glucose levels by the oral starch tolerance test in mice. After the administration of starch, the maximum increase in the blood glucose level was observed at 30 min in all mice. Comparing with control group, mice treated with 250 mg/kg and 750 mg/kg SRPAs exhibited significantly lower blood glucose concentrations ( $p < 0.05$ ). At 60 min, the 250 mg/kg and 750 mg/kg SRPAs groups still showed significantly lower glucose levels than those of the control group ( $p < 0.05$ ). At 120 min, the blood glucose concentrations of all groups recovered to the levels observed at 0 min. These results suggest that SRPAs effectively suppressed the elevation of blood glucose levels after the oral administration of starch in mice. The suppression of blood glucose levels through the inhibition of starch and disaccharide digestive enzymes.

Scarlet runner beans (purple) were processed by pressure cooker, cooked beans and cooking liquid were obtained. Moreover, we added edible brewed vinegar and baking soda during thermal process. Comparing with raw beans, the total polyphenol content (the total polyphenol content value of cooked beans and cooking liquid) and total DPPH radical scavenging activity (the total DPPH radical scavenging activity value of cooked beans and

cooking liquid) were decreased by 31.12% and 66.54%, respectively. The total polyphenol content was increased by 14.15% and 17.59%, respectively, adding with 0.1% and 0.5% brewed vinegar. However, they are significant lower than that of raw beans. The total polyphenol content was decreased by 9.92% and 8.20%, respectively, adding with 200 mg and 400 mg baking soda. The effect of polyphenol content may be a polyphenols in beans decomposition and recomposition after adding with brewed vinegar and baking soda. DPPH radical scavenging activity was increased by 29.69% and 27.35%, respectively, adding with 0.1% and 0.5% brewed vinegar. However, they are significant lower than that of raw beans. DPPH radical scavenging activity was increased by 30.46% and 27.77%, respectively, adding with 200 mg and 400 mg baking soda. Moreover, cooked beans extract residues were treated with 0.1N NaOH solution. Polyphenols were from 0.94 mg/g raw bean to 2.81 mg/g raw bean in cooked beans extract residues of various manufacturing process treatment.

These findings suggest that polyphenols from the seed coat of scarlet runner beans (purple) as a dietary supplement exerting anti-obesity effects through the inhibition of fat digestive enzymes and the suppression of blood glucose levels through the inhibition of starch and disaccharide digestive enzymes. Moreover, the adding of edible brewed vinegar during scarlet runner beans (purple) thermal process could increase the polyphenols and their antioxidant activity of cooked beans.

## 論文審査の結果の要旨

本研究は、花豆（ベニバナインゲン豆）に含まれるポリフェノール成分と抗酸化活性、消化酵素活性の抑制作用についてまとめた論文である。花豆の機能性に関する報告は、これまでにまったくみられない。

まず、試験管内実験により、紫花豆種皮ポリフェノールの特性を解析し、また消化酵素である $\alpha$ -グルコシダーゼ活性と $\alpha$ -アミラーゼ活性およびリパーゼ活性の抑制作用について解析した。続いて、マウスを使った動物実験を実施し、抗肥満作用、抗酸化作用および血糖値上昇抑制作用について解析することにより、花豆ポリフェノールの機能性を明らかにしようとした論文である。さらには、加熱調理による煮豆に含まれるポリフェノールの特性についても解析した論文である。

まず、種皮色の異なる花豆ポリフェノールの特徴を調べ、白花豆以外に豊富にポリフェノールが含まれ、強い DPPH ラジカル消去活性と還元力のあることを明らかにした。食用としてよく利用される紫花豆の種皮と子葉に含まれるポリフェノール割合が約 5 : 1 であり、種皮のそれは、95%以上が (-)-エピカテキンの B 型結合重合体のプロシアニジンであることを明らかにした。次に、紫花豆種皮ポリフェノール (SRPA) には、DPPH ラジカル消去活性、 $\alpha$ -アミラーゼ活性、 $\alpha$ -グルコシダーゼ活性、リパーゼ活性の抑制作用があることを明らかにした。DPPH ラジカル消去活性とリパーゼ阻害活性は、プロシアニジンの重合度に関係があり、重合度が高いプロシアニジンのそれらの活性が強いことも明らかにした。

高脂肪食餌マウスに 1.0%SRPA を与えて 15 週間飼育したところ、体重上昇の抑制、肝臓や脂肪組織の重量の有意な低下、血清の中性脂質量の有意な低下、血清コレステロールの抑制および糞中の脂肪及びコレステロール排泄が認められ、これらの結果から、SRPA に抗肥満効果のあることを明らかにした。また、マウスに SRPA を経口投与して血糖値上昇抑制作用を検討し、SRPA 投与群のマウスの血中グルコース量は、経時的にコントロール群のそれに比べて有意に低い値を示したことから、SRPA 投与がマウスの血糖値上昇を抑制することも明らかにした。

紫花豆を加熱した時のポリフェノールと DPPH ラジカル消去活性に及ぼす影響を検討した。紫花豆生豆から抽出したポリフェノール含量と煮豆から抽出したポリフェノール含量には違いが見られ、煮豆のポリフェノール含量は生豆のその 1/5 であり、煮豆のエタノール、アセトン抽出残渣には抽出されずに残っているポリフェノールが存在すること、抽出残渣の粉末は直接 DPPH 溶液と反応してラジカル消去活性を示すことを明らかにした。0.1%食酢を添加して作った煮豆の DPPH ラジカル消去活性は従来方法で作った煮豆のそれに比べて 1.3 倍で、有意に高いことも明らかにした。

以上により、本審査委員会は、「岩手大学大学院連合農学研究科博士学位論文審査基準」に則り審査した結果、本論文を博士(農学)の学位論文として十分価値のあるものと認めた。

#### 学位論文の基礎となる学術論文

1. Zhaohong Ci, Chengyu Jiang, Shuo Feng, Shan Wu, Yang Cui, Yuki Sasaki, Michiyuki Kojima. (2018). Anti-Obesity Effect of Proanthocyanidins from the Coat of Scarlet Runner Beans on High-Fat Diet-Fed Mice. *Journal of Food and Nutrition Research*. 6(2): 103-109.
2. Zhaohong Ci, Chengyu Jiang, Michiyuki Kojima. (2018). Suppressive Effect of Polyphenols from the Seed Coat of Scarlet Runner Beans on Blood Glucose Levels. *Journal of Food and Nutrition Research*. 6(3): 182-186.
3. Zhaohong Ci, Michiyuki Kojima. (2018). Antioxidant and  $\alpha$ -Glucosidase Inhibitory Activity of Scarlet Runner Bean Polyphenols. *Journal of Food and Nutrition Research*. 6(4): 256-260.