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学位論文題目	Studies on antioxidant effect of yabumame (<i>Amphicarpaea bracteata</i> (L.) Fernald subsp. <i>edgeworthii</i> (Benth.) H. Ohashi) extract (ヤブマメ (<i>Amphicarpaea bracteata</i> (L.) Fernald subsp. <i>edgeworthii</i> (Benth.) H. Ohashi) 抽出物の抗酸化効果に関する研究)
論文審査委員	主査 教授 星 雅之 准教授 新井 博文 准教授 佐藤 利次 教授 吉田 孝 教授 佐藤 之紀

学位論文内容の要旨

Yabumame (*Amphicarpaea bracteata* (L.) Fernald subsp. *edgeworthii* (Benth.) H. Ohashi) is an annual vine in legume family, similar to hog peanut (American wild peanut), and is mainly distributed on the coast of Hokkaido, Japan. The edible portion of yabumame is elliptic underground seeds with dark color spots. Yabumame bean has been consumed as an important traditional food for the Ainu people. However, the bioactivities of yabumame are unknown.

Presently, about 25% of the cause of death in Japan is cardiovascular disease (CVD), including heart disease and cerebro-vascular disease. It is known that onset of CVD is associated with atherosclerosis. It has been suggested that oxidation of low-density lipoprotein (LDL) in the vessel wall by reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and peroxy radicals generated from transition metal ions and lipoxygenase (LOX) may be an early event in development of atherosclerosis, although the mechanisms of LDL oxidation *in vivo* are unclear. It has been studied that the intake of plant polyphenols from foods can reduce oxidative stress which is associated with many kinds of disease including atherosclerosis. In this study, antioxidant effect of yabumame bean extract (YE) on lipid peroxidation and apo B-100 modification in LDL oxidized by peroxy radicals and copper was investigated *in vitro* to elucidate whether yabumame is a candidate food to suppress atherosclerosis. We also analyzed nutritional composition of yabumame beans.

YE was prepared using 80% methanol. Total polyphenol was determined by Folin-Denis method. Radical scavenging activity of YE in solution was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Human LDL was prepared by ultracentrifugation. LDL oxidation was induced by water-soluble radical-generating reagent (2-amidinopropane dihydrochloride, AAPH) or Cu^{2+} in

the presence of YE. As the indices of lipid peroxidation of oxidized LDL (oxLDL), cholesterol ester hydroperoxide and thiobarbituric acid reacting substance were determined by HPLC for free radicals and transition metal ions, respectively. Apo B-100 and protein carbonyls in oxLDL were analyzed using SDS-PAGE and western blotting. Heparin-binding activity of apo B-100 in oxLDL was analyzed by SDS-PAGE with Coomassie Brilliant Blue staining.

Total polyphenol content of YE was 1.9%. YE showed DPPH radical scavenging activity in solution. YE exerted the inhibitory effects on both lipid peroxidation and apo B-100 oxidation in LDL by free radicals and transition metal ions. Although the mechanism is not clear, it can be considered that the antioxidant of YE is due to the radical scavenging polyphenols including isoflavonoids in YE. The present results suggest that yabumame may contribute to preventing LDL oxidation by ROS that leads to atherosclerosis.

Currently, it is estimated that approximately 30% of Japanese people have allergic diseases. Immediate hypersensitivities such as hayfever are classified as type I allergy. Leukotriene (LT) is a chemical mediator in allergic reaction of type I allergy, causing smooth muscle contraction and mucous hypersecretion. LT is produced by LOX reaction, a kind of lipid peroxidation. In recent years, suppression of allergy symptoms by the intake of food components has been attracted much interest because of the low side effects, although taking medicines provides immediate and strong effect in general. Moreover, antioxidants in food may inhibit LOX reaction which produces LT. We also explored effect of YE and its chromatographic fractions on LTB₄ production *in vitro* using mast cells line.

YE was subjected to column chromatography to obtain the chromatographic fractions. LTB₄ production inhibitory activity of YE and its chromatographic fractions were evaluated by using mast cells *in vitro*.

Fractions 17-19 obtained from YE by column chromatography exerted strong LTB₄ production inhibitory activity in mast cells stimulated by calcium ionophore, whereas YE did not suppress the LTB₄ production. We found 7 major unidentified substances in Fractions 17-19. Although the active substances in Fractions 17-19 are not clear, it can be considered that LTB₄ inhibitory activity may be attributed to phenolic compounds such as isoflavonoids which can suppress LOX reaction. Our data indicate that yabumame contains the bioactive phenolic compounds that can prevent LTB₄ production in mast cells which leads to allergy.

In future studies, the structure of the bioactive compounds in Fractions 17-19 and their effects on LDL oxidation and LTB₄ production which are involved in atherosclerosis and allergy should be investigated.

論文審査結果の要旨

活性酸素種による生体酸化ストレスは、さまざまな疾病に関与することが示唆されている。ヤブマメ (*Amphicarpaea bracteata* (L.) Fernald subsp. *edgeworthii* (Benth.) H. Ohashi) は、北海道に多く自生するマメ科一年草であり、アイヌ民族はその地下果を古くから重要な食糧としているが、その生理活性は明らかではない。本論文は、ヤブマメの食品機能の一つとして、活性酸素種に対する抗酸化効果を *in vitro* で明らかにすることを目的としたものである。

ヤブマメ抽出物は、ヒト低密度リポタンパク質の脂質過酸化およびアポリポタンパク質の酸化に対して抑制効果を示した。また、ヤブマメ抽出物の脂溶性画分は、肥満細胞における酵素的な脂質酸化に対して抑制効果を示した。

申請者によるこれらの研究成果は新知見であり、ヤブマメの将来的な健康食品としての利用に資すると考えられる。よって、申請者は、北見工業大学博士 (工学) の学位を授与される資格があるものと認める。