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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation　Effects of Olive Leaf Water Extracts on Physical Properties of Gel Products Prepared from Chicken Meat and Hen Egg （オリーブ葉水抽出物が鶏肉及び鶏卵の加熱ゲル食品の物性に及ぼす影響）

学位論文要約：
Dissertation Summary

Introduction

Chicken gel products are consumed in many countries, especially in Islamic communities, because it is readily applicable to halal food. The chicken gel products' primary material is chicken meat and egg, high-protein food, and well recognized as a healthy product. However, chicken gel products have the drawback of poor textural properties, which negatively affects consumer preference because the texture is an important attribute of consumer preference. The natural plant extract can improve the textural quality of chicken gel products. Hatanaka *et al.*(2009) reported that extracts from three kinds of tea leaf (black tea, oolong tea, and green tea) improve egg white gel's physical strength. The improvements in gel strength differed among the three tea extracts. This result is probably due to the structural difference in catechins, a group of compounds in the flavonoid family, induced by the tea leaf's fermentation. Endogenous enzymes such as polyphenol oxidases are involved in the change in the chemical structure of polyphenol (Moudache *et al.* (2016); Queiroz *et al.* (2008)). Olive leaf is a by-product of olive trees (*Olea europaea* L., Oleaceae) but has a high polyphenol content, so it has the potential to be used as a food ingredient. Olive leaf contains a high amount of polyphenols, which is comparable to the amount in tea leaf. However, the structure of the olive leaf polyphenols is quite different from the flavonoids in tea leaf. The primary polyphenols in the olive leaf are water-soluble non-flavonoid type phenolic compounds such as oleuropein, hydroxytyrosol, and verbascoside (Moudache *et al.* (2016); Rivas *et al.* (2000)). This study investigates the effect of olive leaf water extract powders on the quality of poultry gel products and compares the results of olive leaf extract powder on frozen storage and freeze-thawing of poultry gel products.

Materials and Methods

The olive leaves were dried at 25–40°C for 48 h using a cold-air drying machine (Cool Dry Machinery Co. Ltd.,

Kagawa, Japan) and milled using a grinder. The resulting powder was sieved using a nylon mesh filter (108 μm mesh diameter). Two olive leaf water extracts were prepared at 4 and 80°C from the sieved olive leaf powder. For the 4°C and 80°C extractions, 50 g of the olive leaf powder was mixed with 500 mL of pure water, which was set at an extraction temperature in advance, in an Erlenmeyer flask and agitated using a magnetic stirrer for 1 h at 4°C. Each of the resulting homogenates was centrifuged at 8000 × *g* and 4°C for 20 min. The supernatant was lyophilized. The lyophilized powder was stored at −20°C until further experiments.

The powder of olive leaf water extracted at 4°C was named OEx_4, and that of water extracted at 80°C, OEx_{80}. The two olive leaf water extracts, OEx_4 and OEx_{80}, have been analyzed to the chemical characteristic using the proximate analysis, the polyphenol content, the antioxidant activity, and the primary phenolic constituents. The primary phenolic components of the two extracts were analyzed using reverse-phase HPLC and LC/MS.

The olive leaf water extracts (OEx) powder have been applied to the CBS, WE (Whole Egg), and EW (Egg white). The CBS-OEx, WE-OEx, and EW-OEx were analyzed the physical (breaking strength, viscoelasticity, water holding capacity (WHC), and microstructure observation analysis) and chemical properties (determination of cross-link pre-treatment).

The effects of the addition of OEx on the quality deterioration of CBS caused by frozen storage were investigated (thawing loss, breaking strength, viscoelasticity, WHC, lipid oxidation, whiteness, and polyphenol determination).

The effects of the addition of OEx on the quality deterioration of WE and EW caused by frozen storage were investigated (thawing loss, breaking strength, viscoelasticity, and WHC)

Results and Discussion

Chemical Characteristics of Olive Leaf Water Extract Powder

The chemical characteristics of phenolic compounds extracted from the olive leaf using water set at two different temperatures (4 and 80ºC) were investigated. There are no significant differences in pH, sugar content, and salt content between OEx_4 and OEx_{80}. Protein content slightly differed between OEx_4 and OEx_{80}, the protein content of OEx_4 containing 140.4 mg/g dry weight (DW), which was 2.4% lower than that of OEx_{80}. The polyphenol content differed between OEx_4 and OEx_{80}, with OEx_4 containing 138.5 mg GAE/g DW, which was 32% lower than that of OEx_{80}. The oleuropein content of OEx_4 was 22.10 mg/g DW, which was 8-times lower than that of OEx_{80}. These results indicate that OEx_4 had much lower polyphenol and oleuropein contents than OEx_{80}, probably due to the actions of endogenous enzymes β-glucosidase and polyphenol oxidases in the olive leaf. The 4°C-extraction process of the olive leaf powder did not inactivate the oxidation enzymes in the olive leaf, while

the 80°C-extraction process must have partially inactivated those enzymes (Chen *et al.,* 2016). The antioxidant activity of OEx_4 was 1.22 mmol TEAC/g DW, which was 12% lower than that of OEx_{80}. Thus, polyphenol extraction using cold water appears to lower the antioxidative function of olive polyphenol.

In the OEx_{80} chromatogram, there is a prominent peak at 50.29 min. The peak had a major fragment ion at 539 m/z, which was assigned to oleuropein. There were several other peaks at 46.70, 49.43, and 52.11 min, which were assigned to hydroxyoleuropein (555 m/z), the decarboxymethyl form of 3,4-DHPEA-EDA (319 m/z), and oleuroside (539 m/z), respectively. The phenolic compounds seen in OEx_{80} were the same as the major phenolic compounds extracted from olive leaf using 60% ethanol (Mylonaki *et al.,* 2008). The HPLC chromatogram of OEx_4 was dissimilar to that of OEx_{80}, showing that oleuropein drastically decreased, while the decarboxymethyl form of 3,4-DHPEA-EDA was a major component. Hydroxyoleuropein (46.95 min) and oleuroside (50.44 min) were also present in OEx_4. However, their peak areas were lower than those of the corresponding peaks of OEx_{80}, suggesting that the abundance of those phenolic compounds was lower in OEx_4 than in OEx_{80}. These results indicate that olive leaf endogenous enzymes modified the chemical structure of the polyphenols during the extraction process of OEx_4.

HPLC analyses showed that a major phenolic compound of OEx_{80} is oleuropein, the most abundant olive leaf polyphenol; while that of OEx_4 was an aglycone form of oleuropein called 3,4-DHPEA-EDA. The aglycone probably has been generated by the action of leaf enzymes (β-glucosidases) during extraction using water at the low temperature of 4°C.

Effects of OEx on Quality of Chicken Breast Sausage

The two OExs (OEx_4 and OEx_{80}) with different phenolic compositions were applied for manufacturing CBS, and the resulting OEx-containing CBS's were evaluated for their chemical and physical properties. Furthermore, the effects of OEx_4 on the quality deterioration of CBS caused by frozen storage were investigated. Using the olive leaf water extract OEx_4 as an additive significantly modified the mechanical and viscoelastic properties and WHC of CBS. Changes in the physical properties of CBS are generally accompanied by modifications in muscle proteins, especially myosin. We were investigated the effects of OEx_4 on the thermal and chemical properties of meat proteins. The control meat batter showed three major endothermic peaks at 53.1, 62.5, and 69.0°C correspondings to the denaturation of myosin, connective tissue (together with sarcoplasmic proteins), and actin, respectively (Li *et al.* (2015); Li *et al.* (2015)). The meat batter containing 0.1% OEx_4 had peak temperatures similar to that of control.

In contrast, the 0.3 and 0.5% OEx_4 meat batters had peak temperatures of the first peak 1.9 and 2.7°C higher than

control, respectively, suggesting that a substantial amount of OEx_4 retards thermal denaturation of myosin. The enthalpy (ΔH) of the myosin peak for control, 0.1, 0.3, and 0.5% OEx_4 meat batters was 0.452, 0.378, 0.309, and 0.296 J/g, respectively, implying that the denaturation ΔH attributed to myosin decreases with increasing OEx_4. These results indirectly indicate that some component of OEx_4 interacts with myosin and affects the thermal transition of myosin. The gel strength of proteinous gel is closely associated with surface hydrophobicity of protein (Balange and Benjakul (2009); Zhang *et al.* (2015)). Consequently, the protein surface hydrophobicity of natural actomyosin (NAM) was analyzed. The surface hydrophobicity of NAM treated with OEx_4. The *S*o value increased as the OEx_4 concentration increased. NAM treated with 0.5% OEx_4 had 4.5-times higher surface hydrophobicity than that without OEx_4 (control). Thus, OEx_4 is found to enhance the surface hydrophobicity of NAM. The effects are primarily due to the polymerization of the meat proteins induced by OEx_4. Non-disulfide type covalent bonds appear to be involved in the polymerization. To explore which functional groups of NAM proteins are involved in the protein polymerization, the NAM's sulfhydryl and primary amino groups were determined. The sulfhydryl (SH) content of NAM decreased significantly with increasing OEx_4 concentration, and a marked drop occurred with 0.1% OEx_4 (Fig. 3.6). The primary amino group of NAM also declined with increasing OEx_4 concentration. Adding 0.5%, OEx_4 resulted in a 36% reduction in the total amino group. The two functional groups' results strongly suggest that OEx_4 compounds interact with the thiol group of the cysteine residues, the ε-amino group of lysine residues, and/or the α-amino group of the meat proteins. Considering that OEx_4 promotes the formation of a non-disulfide covalent bond between meat proteins, key molecules in OEx_4 probably cross-link proteins by covalently binding to the SH group and amino group of proteins.

The application of OEx_4 to CBS was effective in suppressing physical property changes (syneresis and texture deterioration) and chemical property changes (lipid oxidation and discoloration) induced by frozen storage. The physical property changes were suppressed by the cross-links between CBS protein molecules induced by 3,4-DHPEA-EDA, a major phenolic compound, in OEx_4. The chemical property changes were suppressed by some antioxidants (phenolic compounds) contained in OEx_4. From the above results, this natural OEx_4 is useful in improving the quality of frozen meat products.

Effects of OEx_4 on Physical Properties of Egg Gel

The OEx_4 showed improved physical properties of CBS gel and prevented deterioration from frozen storage. This research aimed to examine the physical properties of WE and EW gels containing OEx_4 and the effects of OEx_4 on freeze-thawing of WE and EW gels. OEx_4 showed the enhancements in the physical properties (WHC, breaking strength, and viscoelasticity) of the egg gels, although the effect of OEx_4 to WHC was larger for WE gel

than for EW gel. The preventing effects of OEx_4 on gel's physical deterioration by frozen storage were investigated by freeze-thaw abuse of the gels. Thawing-loss, breaking strength change, and viscoelastic changes of WE and EW gels caused by freeze-thaw abuse were highly suppressed by the addition of OEx_4.

The microstructural images of unfrozen and three-times freeze-thaw repeated WE gels. The surface of the unfrozen WE gels with/without OEx_4 was smooth. On the other hand, the images of freeze-thawed gels of control and +0.03% OEx_4 were indicative of a coarse texture with large cavities (5 to 30 μm). The control gel was particularly seriously damaged, and its cavities were larger than that of 0.03% OEx_4-gel. In the freeze-thawed, 0.1% OEx_4-gel were not observed such cavities. The damage in the microstructure of EW gel was different from the case of WE gel. The difference was as follows: the surface of three-times freeze-thawed control EW gel was coarser and had large cavities; while that of 0.03% OEx_4-gel was still smooth and did not have large cavity, even though being slightly coarser than the unfrozen OEx_4-egg gel. Such cavities were not seen in the three-times freeze-thawed 0.1% OEx_4-gel. These minimal morphological changes in the OEx_4-egg gels support the physical resistance to freeze-thaw cycle seen in the breaking strength and creep analysis results. Also, the high frozen tolerance of the OEx_4-gels can be linked to the stabilization of water behavior of egg gel.

SDS-PAGE pattern of protein extracted from the OEx_4-WE solution that was heat-treated. The control WE proteins showed 73kDa α-livetin, 59kDa phosvitin, and 36kDa β-livetin, derived from egg yolk (EY), and 75kDa ovotransferrin, 45kDa ovalbumin, 14kDa lysozyme, which are derived from the egg white. In the SDS-PAGE pattern of OEx_4-WE solutions, many bands derived from both EY and EW disappeared, and the remaining bands also became thinner than the corresponding bands of control WE proteins. The α-livetin and ovotransferrin disappeared, and that of ovalbumin, lysozyme, phosvitin, and β-livetin became thinner. Moreover, a new band appeared at the top of the stacking gel, suggesting that the addition of OEx_4 formed high molecular protein complexes. The disappearance of protein bands and the high molecular weight band's concomitant appearance suggest that OEx_4 induced protein cross-link as was seen in the protein matrix of CBS.

SDS-PAGE analysis of the EW proteins reacting with 0.03% and 0.1% OEx_4 showed changes in ovotransferrin (75kDa), ovalbumin (45kDa), and lysozyme (14kDa). The ovotransferrin and lysozyme bands disappeared, and the ovalbumin band became thinner. This implies that non-disulfide-type intermolecular-crosslink occurred among ovotransferrin, lysozyme, and ovalbumin, resulting in polymerization formation. The cross-links between egg protein molecules suppressed the physical property changes, anti-syneresis, and frozen tolerance by use of OEx_4.

Conclusion

In this study, the two olive leaf water extracts OEx_4 and OEx_{80} that were prepared at different extraction temperatures (4 and 80°C) have been applied to the CBS. For the CBS, OEx_4 and OEx_{80} both showed the enhancement of physical properties, but the enhancing effect was much higher in OEx_4 than in OEx_{80}. The effectiveness of OEx_4 was more noticeable when the gel products were frozen-stored. OEx_4 showed excellent tolerance against various deteriorations of CBS, WE gel and EW gel occurring during frozen storage, such as drip loss and physical changes. These superb effects are due to the phenolic compounds of OEx_4, of which major compound is an aglycone form of oleuropein called 3,4-DHPEA-EDA. The aglycone probably has been generated by the action of leaf enzymes (β-glucosidases) at the low extraction temperature of 4°C. It is suggested that 3,4-DHPEA-EDA made chicken gel products more compact gel network because of its high protein crosslinking activity. We propose that the modification of the gel network resulted in the improvement of physical properties, including the effectiveness in frozen tolerance.

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