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研究課題名（和文）Development of antimicrobial packaging system foreseeing enhanced storage stability of lotus root (Nelumbo nucifera)

研究課題名（英文）Development of antimicrobial packaging system foreseeing enhanced storage stability of lotus root (Nelumbo nucifera)

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研究成果の概要（和文）：レンコンの酸性加工ならびに温度、酸素濃度等の異なる貯蔵条件下で品質を保つことを目的として、フレッシュカットしたレンコンをクエン酸の水溶液に浸し、色差変化、硬さ、ポリフェノールオキシダーゼの酵素活性を測定し、レンコンにおける品質評価の検討を行う。
又、抗菌物質であるナイシンおよびカルバクロールを 内包したO/Wナノエマルションの調整条件、安定性等の検討を行う。次に、食品包装への応用を目指した生分解性フィルムの作製条件の検討に併せて、抗菌活性を持つエマルションを、生分解性フィルム形成の溶液と混合し、抗菌性の生分解性フィルム作製の検討を行う。

研究成果の概要（英文）：The overall purpose of this research is to enhance the storage stability of lotus root. In order to achieve this goal, in the first year of the project, on one hand we focused on the development of composite films consisting of different combinations of chitosan and nanoclay as a primary step to produce films with enhanced mechanical properties and selective gas barrier, foreseeing their application as packaging material for lotus root, and possibly reduce the browning upon storage.
We also looked into the development and characterization of Oil-in-Water nanoemulsions loaded with natural antimicrobial and antioxidant compounds, for optimum antimicrobial and antioxidant activities. The experimental results indicated that the O/W nanoemulsions loaded with both, nisin and carvacrol, having droplet mean diameter of around 120 nm, were considerably stable under the conditions investigated (up to 1 week storage at room temperature).

研究分野：　Agricultural Sciences

キーワード：　Food Engineering

１．研究開始当初の背景

(1) The consumption of fresh foods such as horticultural products, seafood, and meat has increased considerably over the past decades, due to the need of consumers for convenient ready-to-eat or ready-to-cook foods, along with their desire to have a healthy lifestyle. One of the major challenges for food manufacturers is the limited storage life of these fresh foods, coupled with their association to foodborne disease outbreaks, resulting in continuing commercial pressures to use synthetic chemicals as preservatives.

(2) At present, a wide variety of chemical preservatives are permitted and used in foods to prevent the growth of food spoilage and disease causing bacteria. The type of preservative, amount allowed to be used and in which foods vary between countries but use of preservatives is increasingly being negatively perceived by consumers. On the one hand, the use of plant antimicrobials fulfil the needs of today's consumer looking for wholesome food without chemical preservatives. Nevertheless, the antimicrobial activity of plant extracts that is observed in in vitro conditions is different from effect in complex food systems. In most cases, antimicrobial activity is decreased due to interactions with food components. On the other hand, essential oils comprise a large number of components, and their mode of action is likely involved with several targets in the bacterial cell. Their hydrophobicity enables them to partition in the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents.

(3) In this regard, we have been conducting research on the development of Oil-in-Water (O/W) nanoemulsions loaded with nisin, a hydrophilic compound with antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis*, and carvacrol, an essential oil from oregano (*Origanum vulgare*). We have investigated those antimicrobials separately, or the synergistic effect between them when loaded into O/W emulsions. The combination of nisin (dissolved in the aqueous phase) and carvacrol (dissolved in the oil phase) into O/W nanoemulsions resulted in the antimicrobial activity against spores of *B. subtilis*, but the synergistic mechanism has not been completely understood yet. Moreover, different process parameters various conditions for the formulation of emulsions containing droplets of different mean diameter should be tested, in order to compare their antimicrobial potential

２．研究の目的

The ultimate goal of this research is to extend the shelf life of minimally processed lotus root (*Nelumbo nucifera*), foreseeing their shipment to other regions within Japan, and more especially for export this high-quality specialty to overseas markets.

３．研究の方法

In order to reach our final goal, this research was basically divided into three major tasks, as follows: Task 1: Development of Oil-in-Water nanoemulsions loaded with hydrophilic and hydrophobic natural antimicrobial compounds, more specifically nisin and carvacrol respectively, investigating the synergistic effect between them. Task 2: Development edible coatings and films, as package for fresh-cut lotus root (*Nelumbo nucifera*). Task 3: Optimization of the antimicrobial properties of the coating developed.

４．研究成果

In the first of the project, on one hand we focused on: **1)** The development of composite films consisting of different combinations of chitosan and nanoclay as a primary step to produce films with enhanced mechanical properties and selective gas barrier, foreseeing their application as packaging material for lotus root, and possibly reduce the browning upon storage. **2)** We also looked into the development and characterization of Oil-in-Water nanoemulsions loaded with natural antimicrobial and/or antioxidant compounds, for optimum antimicrobial and antioxidant activities. Ideally, such antimicrobial nanoemulsions may be incorporated into edible coatings and/or nanocomposite films with antimicrobial properties, to be used as package for fresh-cut lotus root. To this end, we evaluated the encapsulation of two antimicrobial compounds, nisin and carvacrol, into Oil-in-Water nanoemulsions, and monitored their antimicrobial activity against *Bacillus subtilis*, an undesirable food pathogen. The experimental results indicated that the O/W nanoemulsions loaded with both, nisin and carvacrol, having droplet mean diameter of around 120 nm, were considerably stable under the conditions investigated (up to 1 week storage at room temperature). In addition, a reduction of nearly two log cycles (around 100 times) on *B. subtilis* population was achieved upon treatment with O/W nanoemulsions loaded with both antimicrobial compounds, compared to the blank (10 mM phosphate buffer), upon incubation for 24 h.

In the second year of the project, we focused on: **3)** The development of antimicrobial nanoemulsions, foreseeing the development of antimicrobial films foreseeing their usage as package for shipping fresh-cut lotus root to long distance. For this purpose, we investigated the

antimicrobial activity of essential oil from *Thymus capitatus*, either in bulk form or encapsulated into Oil-in-Water nanoemulsions, and monitored their antimicrobial activity against *B. subtilis* and *Escherichia coli*, an undesirable food pathogen. The experimental results indicated that either bulk or nanoencapsulated *T. capitatus* essential oil was able to preserve the quality and enhance the shelf life of food products. **4)** We also looked into the development of technology for reducing the browning of fresh-cut lotus root upon storage, such as the effect of low pH treatment, and storage at reduced pressure. In order to achieve this goal, during storage of fresh-cut lotus root we monitored the changes in color, texture, micro-structure and the enzymatic activity of polyphenol oxidase, as it plays a major role in overall quality loss due to oxidation upon exposing lotus root to oxygen. The experimental results indicated that fresh-cut lotus root treated with citric acid aqueous solutions at different pH (ranging from pH 2 to pH 4), had the lowest change in color up to 8 days of storage under refrigeration, indicating reduced browning activity.

In the third year of the project, we focused on: **5)** Potential use of different essential oils, e.g. from *Thymus capitatus*, to be used as natural preservatives. For this purpose, we investigated about the formulation and characterization of Oil-in-Water nanoemulsions encapsulating Thymus essential oil. Seemingly, their physical stability and antibacterial efficiency were also monitored upon storage at different conditions. **6)** The authors also investigated about the incorporation of xanthan gum or guar gum in the emulsion system, as strategy to enhance the physical stability of such essential oils loaded into nanoemulsions, considering the low viscosity of essential oils in general - in some cases, close to that of water, which is a critical point to attain stable emulsions loaded with such essential oils. The experimental results indicated that nanoencapsulated *T. capitatus* essential oil was able to preserve the quality and enhance the shelf life of food products, without perception of its undesirable scent by a sensory panel, unlike the bulk essential oil. We also looked into the effect of coating fresh-cut lotus root with a single layer coating, or layer-by-layer coating using guar gum, xanthan gum or chitosan, and investigated their respective effect on the storage stability of fresh-cut lotus root.

５．主な発表論文等

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６．研究組織

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