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## Limonene encapsulation in alginate/poly (vinyl alcohol)

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### Abstract

Aroma is one of the most important characteristics of food products. Substances used as aromas are usually composed of many volatile and odorous organic species. They are usually thermally sensitive chemical compounds, which require special treatment during food processing. Consequently, the degradation of aroma compounds during production, storage and/or transport could be critical in terms of stability and quality. Encapsulation might be one of the methods which could be used in order to improve aroma quality and stability. The aim of this study was to create a stable alginate/polyvinyl alcohol formulations, which could be used in order to protect and improve stability of liquid aroma compounds such as d-limonene (4-isopropenyl-1-methylcyclohexene). Encapsulation of d-limonene in calcium alginate/polyvinyl alcohol matrix was realized by “freezing-thawing” method in order to assure formation of polyvinyl alcohol cryogel structure. Afterwards, samples were immersed in calcium chloride solution for calcium alginate gel formation. In order to investigate thermal decomposition of alginate/polyvinyl alcohol matrix and kinetics of aroma release, the samples of encapsulated d-limonene were investigated by thermogravimetry (TG). The initial aroma concentrations were in the range 1-10% w/w, while the polyvinyl alcohol concentration varied in the range 5-20% w/w. It was found that thermal properties of encapsulated aroma depend on thermal properties of the matrix. Maximum weight loss of free d-limonene occurred at about 120°C, while in a case of encapsulated aroma, weight loss occurred at higher temperatures. The results indicate better stability of the encapsulated d-limonene in comparison with free aroma. Therefore, alginate/polyvinyl alcohol system can be considered as suitable for aroma encapsulation.

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## 1. Introduction

The preservation of aromatic food ingredients, which are not only delicate and volatile, but also very expensive, is a complex problem. Encapsulation could be an adequate solution for aroma protection from evaporation and degradation [1]. For successful aroma retention and protection, it is important to choose an appropriate shell material as well as encapsulation technique. Alginates are one of the best known materials used for the encapsulation of a wide variety of aroma compound [2]. The alginates are linear polysaccharides extracted from brown seaweeds. In the natural environment, alginates exist as  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  salts of alginic acid. They contain different amounts of (1-4)-linked  $\beta$ -D-mannuronic acid (M) and  $\alpha$ -L-guluronic acid (G) residues. The amounts as well as arrange of residues determine the physical properties of the alginate. The ability of alginate to form gels in the presence of some multi-valent metal ions is one of the most important properties of this group of polymers. Based on this property, alginates have been used extensively as gel-forming agents in food and pharmaceutical products [3].

Poly(vinyl alcohol) or PVA is an odourless and tasteless, white or cream coloured granular powder. It is soluble in water, slightly soluble in ethanol, but insoluble in other organic solvents. Polyvinyl alcohol protects the active food compounds from moisture, oxygen and other environmental influences. This polymer has various applications in food industries as a coating agent. It has been used as a film coating agent, especially as a component of food supplement tablets. One of the most promising applications of this polymer in food industry is active packaging. The active packaging is based on active role of coating materials in food protection. The film on the surface of the product could be used as a carrier for controlled release of active compounds. Those active compounds may have antimicrobial, antioxidant or others important properties [4]. Poly(vinyl alcohol) gels could be formed by several methods, such as chemical crosslinking [5] or by applying freeze-thawing procedure [6].

Polymer blending is an important approach to improve physical properties of a shell material. Alginate/PVA blend combines advantages of both: poly(vinyl alcohol) hydrogels have good mechanical properties, while alginates are suitable for preparation of stable encapsulated forms, like spheres [7,8]. Blends of PVA and alginate have been used mainly as carrier materials for immobilizing enzymes and cells [7-9], sorption studies [10] and separation processes [11].

In this paper, the preparation and thermal properties of calcium alginate/PVA composite hydrogel loaded with d-limonene were described. Compositions of polymer blends as well as limonene concentrations were varied in order to provide a stable system. The results of thermogravimetric analysis of samples with d-limonene were compared with those for free aroma.

## 2. Materials and Methods

In this study, a low viscosity sodium alginate (from *Macrocystis pyrifera*) and poly(vinyl alcohol) (Av. Mol. Wt. 30,000-70,000, hot water soluble), both purchased from SIGMA were used. D-limonene (minimum 97%) was supplied by HiMedia Laboratories Pvt.Ltd. (Mumbai, India). Calcium chloride dihydrate was purchased from Analytica (Czech Republic).

### 2.1 Preparation

Sodium alginate powder was dissolved in distilled water to prepare 5% w/w solution. PVA was dissolved in distilled water at 70°C to produce solution of concentration 10% w/w. Sodium alginate and PVA were mixed on magnetic stirrer in: 95:5; 90:10 and 80:20 ratio. Initial limonene concentrations were 1, 5 and 10% w/w. The mixtures of polymers and aroma were prepared on a magnetic stirrer at room temperature. The final samples were placed in glass plates (50 mm x 10 mm). The gels were frozen for 12h and thawed slowly in one step in order to provide PVA cryogel formation. Thawed gels were

immersed in calcium chloride solution (0.015 g/ml) in order to provide calcium alginate gel formation. As-formed hydrogels were washed with distilled water and air-dried at room temperature to a constant weight.

## 2.2. Thermal analysis

The thermal properties of alginate/PVA films were investigated employing the simultaneous TG/DSC/MAS technique using Setaram's system TG/DSC111 coupled with mass spectrometer (Thermostar from Pfeifer). All experiments were carried out under dynamic helium of a flow rate of 15 ml/min (pressure 1 atm) using a heating rate of 5°C/min (25-200°C temperature range) per run.

## 3. Results and Discussion

In this study, d-limonene was immobilized within alginate/PVA blend matrix. Alginate was added to improve the final shape and mechanical stability of the matrix. The primary process in gel formation was gelation of PVA by freeze-thaw method, followed by gelation of sodium alginate in the presence of Ca<sup>2+</sup> ions. It was found that 20% w/w of PVA in polymer blend is an optimal concentration for stable gel production. At lower PVA concentrations, the leakage of sodium alginate occurred. Alginate/poly(vinyl) alcohol gel prepared by freeze-thaw method appeared to be stable and therefore could be used in real food processing.

The influence of d-limonene on gel formation and stability was also studied. The initial aroma concentration was found to be critical for encapsulation efficiency. At low initial concentration of limonene (1% w/w), the leakage of aroma was not observed. However, at higher limonene concentrations (5 and 10% w/w), significant release of aroma occurred. Released d-limonene was collected at the bottom of glass plate and detected visually as aroma loss. One possible reason for low retention of d-limonene is hydrophobic nature of this aroma, which led to separation of mixture polymer/aroma into two layers. The other reason for this might be inefficient mixing and dispersion of aroma inside the gel. Kaushik and Roos [12] suggested that homogenization combined with appropriate polymer composition is critical for high retention of limonene during preparation of samples before encapsulation.

The results of thermal stability of free d-limonene and empty carrier are shown in Figure 1. According to our results, temperature range from 60 to 130°C is related to free limonene evaporation. Total residue at 130°C was 1.93%. Results presented by Figure 1 express some differences in evaporation kinetics for free limonene compared with literature data. For example, Hazra et al. [13] reported similar thermal properties of limonene under the atmosphere of nitrogen. According to our measurements, the bulk of limonene evaporated in somewhat lower temperature range in comparison with was found by Hazra et al. Those variations in thermal properties of free limonene could be explained by differences in experimental set-up or by chemical composition of aromas used in experiments. The TG curves of the calcium alginate/poly(vinyl alcohol) (20:80) mixture (with 1, 5 and 10% w/w of limonene), monitored in the 25-200°C temperature range, are shown in Figure 2. Weight losses for samples A, B and C at 130°C were 91.27, 90.83, and 91.71%, respectively; and occur in the whole applied temperature range. According to Parikh and Madamwar [14], thermal decomposition of polysaccharides includes several processes like dehydration, depolymerization and formation of the aromatic and graphitic carbon structures. Temperature range applied here (25-200°C) was selected since cooking process is usually performed in this particular range. This temperature range corresponds to the loss of different types of water from calcium alginate gel. Manojlovic et al. [3] reported that decomposition of the calcium alginate begins around 155°C, while about 20 % of the matrix decomposes up to 230 °C. Holland and Hay [15] reported that pyrolysis of PVA happen in temperature range between 200 and 300°C, the major product is water.

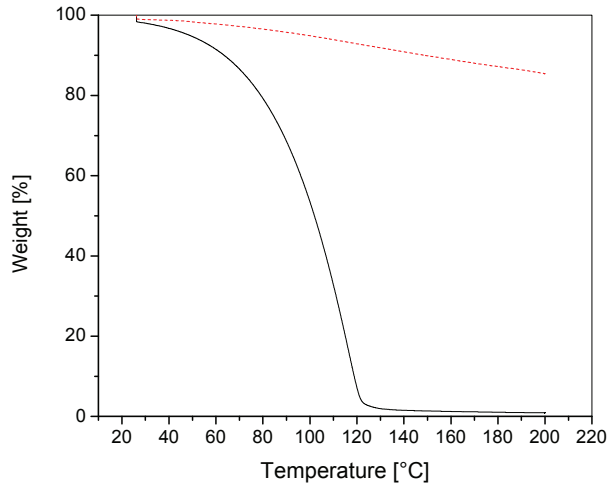


Fig. 1. Thermogravimetric analysis of free d-limonene (solid line) and empty carrier (the calcium alginate/poly(vinyl alcohol) (20:80)-dash line)

Differences in the kinetics of d-limonene evaporation from the mixture with the used matrix can be noticed, in comparison with that one found for pure aroma. It is important to notice that in the course of programmed heating only the fragmentations of d-limonene and alginate were monitored in the detector of mass spectrometer. Results presented in Figure 2. indicate that in the case of encapsulated d-limonene, its release proceeds gradually at a constant rate, without rapid weight losses as in case of free aroma. It can be inferred that, as a result of encapsulation, thermal stabilization of d-limonene happened.

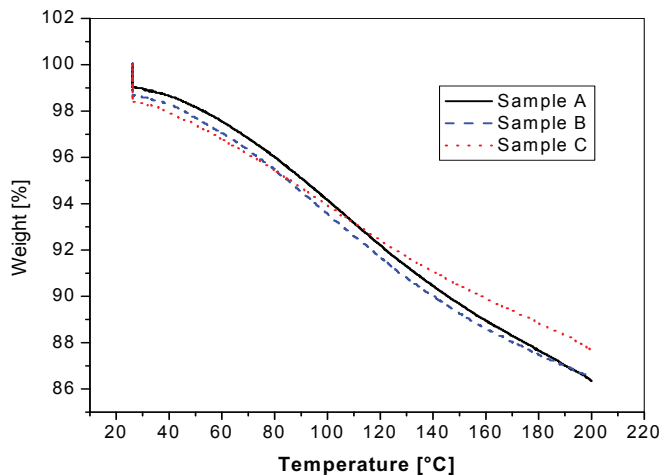


Fig. 2. Thermogravimetric analysis of encapsulated d-limonene. The initial aroma concentration was: Sample A-10% w/w; Sample B-5% w/w; Sample C-1% w/w.

#### 4. Conclusion

Calcium alginate/poly(vinyl alcohol) mixture was found to be an efficient encapsulation matrix for limonene encapsulation. The results of this study suggest that alginate/poly(vinyl alcohol) (20:80) mixture could be used for limonene encapsulation by applying freeze-thaw method followed by alginate gelation via ionic interactions between polymer and gelating ions. The results of thermal analyses showed that encapsulated d-limonene release in extended temperature region in comparison to free aroma. However, leakage of aroma, especially at higher concentrations (10% w/w) appeared to be the main problem. Better retention of limonene might be achieved by efficient homogenization; this will be the subject of further studies.

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