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# The improvement of propionic acid safety and use during the preservation of stored grains

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ABSTRACT

The current study deals with the development of an innovative method in which propionic acid (PA), the common fungistat, has been applied to dry agricultural products not as a liquid but as part of an encapsulated delivery system in order to ensure a safer application and improve its fungistatic activity. Various delivery systems were formulated and included biodegradable polymers as platforms and β-cyclodextrin (β-CD) as an encapsulating agent. The prepared encapsulation systems were characterized for their physical properties and applied to wheat grains at different formulation compositions, dosage levels and applied methods. It was found that encapsulated PA in carboxymethyl cellulose (CMC)-based films with β-CD demonstrated the best fungistatic activity among the prepared formulations. Results from this study indicate for the first time that encapsulated antifungal active agents may have the potential to serve as effective and safe antimicrobial formulations in agricultural products, leading to improved storability and quality with recyclable multi-purpose abilities.



## 1. Introduction

Fungi growing on stored grains can cause much damage, leading to quality reduction and consequential economic losses. Fungal damage can be expressed by heating, nutritional losses, discoloration, and mycotoxin production, which is highly dangerous to both human and animal health (Christensen and Meronuck, 1986; Axel et al., 2016). Fungal growth can be inhibited either by physical means (cooling, modified atmospheres, gamma irradiation), plant extracts (especially essential oils extracted from herbs and spices), biological control (using yeasts and bacteria), and integrated control, which is a combination of different control methods (Farkas, 2007; Cowan, 1999; Barka et al., 2002). Although these means are successful, they are not commonly used, mainly due to several disadvantages, *e.g.*, fungistatic rather than fungicidal effect and a limited period of activity. Physical means are often accompanied by high costs, plant extracts require high dosages for a successful inhibition and a biological antagonist requires special care in its choosing along with difficulties in applying it to grains. As a consequence, fungi-inhibiting chemicals (mainly low molecular weight organic acids like propionic acid (PA), acetic acid (AA) and their salts) designated as Generally recognized as safe (GRAS) by the FDA (Code of Federal Regulations, 2017) are still common fungistats widely used to preserve grain and animal feeds.

PA is the most common commercial grain preservative used as a fungal growth inhibitor and has been utilized for many years as a fungistat by directly adding it to various stored agricultural products (Brul and Coote, 1999; Mani-Lopez et al., 2012). However, its common application as a liquid leads to many disadvantages. The acid is corrosive to metal containers and handling it requires special attention in order to minimize exposure. Its use does not guarantee a uniform dispersion, which is required for maximum effect and depends on the grains' moisture content. Most importantly, PA's fungistatic activity is known as time limited and its treatment is therefore suitable for storage periods of a few weeks or months (Christensen and Sauer, 1982; Woolford, 1975; Tzatzarakis et al., 2000).

It is therefore necessary to develop novel technologies that will reduce PA's disadvantages and better its efficiency. Specifically, in terms of longer antimicrobial time frames and safer applications. An active agent's efficiency may be improved by using

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encapsulation systems, which allow for its controlled accumulation and release (Rashidi and Khosravi-Darani, 2011; Pothakamury and Barbosa-Canovas, 1995). Encapsulation of volatile molecules has been shown to help reduce their volatility, improve their efficiency, mask undesired accompanying odors and aftertaste, and protect them from external factors such as light, oxidation and heat (Lakkis, 2007; Han et al., 2008; Pan et al., 2013; Teng et al., 2012).

Biodegradable active films composed of natural polymers are used for storage and controlled release of antimicrobial agents, as they provide an advanced protection strategy for their constituting ingredients (Cha and Chinnan, 2004; Baldwin et al., 2011; Indrani et al., 2011). Chitosan is a biodegradable polymer yielded by the deacetylation of chitin (a natural polysaccharide). This biopolymer is unique in that it possesses significant antimicrobial abilities, making it widely utilized in food products, as well as biomedical research and applications (Dutta et al., 2009; Elsabee and Abdou, 2013; Luo et al., 2012). Cellulose derivatives are renewable, widely available, eco-friendly hydrocolloids that have a wide range of applications (Siro and Plackett, 2010; Malafaya et al., 2007) and have also been utilized in the past as biodegradable systems for controlled release of antimicrobial agents (Han, 2003; Li et al., 2008).

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide and contains both a hydrophobic cavity and a hydrophilic exterior surface (Astray et al., 2009). This combination, along with its structural properties, make it ideal for transporting hydrophobic molecules in aqueous environments by encapsulating them in its cavity for a range of purposes (Del Valle, 2004). An active agent/β-CD host/guest complex can also be transported in a natural biopolymer-carrying matrix (Moya-Ortega et al., 2012). Moreover, β-CD was recently discovered to successfully increase PA uptake in biopolymer-based films, despite the latter being classified as hydrophilic (Rutenberg et al., 2016a,b).

There is currently no published work dealing with fungal inhibition by an encapsulated active agent in a dry agricultural product. The current study examines for the first time PA's efficacy when applied in an encapsulated form on fungal growth in wheat grains. This novel method allows PA's fungistatic abilities to be amplified, allowing for a prolonged antifungal effect and leading to improved storage quality and safety for wheat grains. Other positive ramifications include smaller economic losses during the postharvest stage and less direct exposure between the food products and the corrosive acid. This safe approach has the potential to be incorporated into practical use of managing mold inhibition in postharvest agricultural products and may lead to a more effective control.

## 2. Materials and methods

### 2.1. Materials

All reagents were of analytical grade and used without further purification. Carboxymethyl cellulose sodium salt (CMC), propionic acid (PA), and phenolphthalein 1% w/v solution in alcohol were purchased from Alfa Aesar (Heysham, England). Chitosan was purchased from Molekula (Newcastle, England). β-CD was purchased from Chem-Impex Int'l Inc. (Wood Dale, IL, USA). Calcium propionate was purchased from Sigma Aldrich (Rehovot, Israel). Sodium hydroxide pellets were purchased from Merck KGaA (Darmstadt, Germany).

### 2.2. Film preparation

CMC-based films were prepared by dissolving 15% w/v PA in double distilled water (DDW). The solution was then heated to 50 °C with a stopper over the flask's top. Next, 5% w/v β-CD was added and stirred for 1 h. In case of formulations without β-CD, this stage was skipped. 2% w/v CMC was then added and the reaction was stirred for 2 h at 50 °C. All films were obtained by pouring 9 mL portions of the film forming solutions into Teflon Petri dishes (9 cm in diameter). All films spontaneously dried at 23 °C overnight in a chemical hood at relative humidity (RH) of 65 ± 2%. The prepared films were stored at −20 °C until their application in the experiments. Chitosan-based films were prepared by dissolving 15% w/v PA in DDW. In case of formulations without PA, 0.6% w/v acetic acid was added to the aqueous solution. The solutions were then heated to 50 °C with a stopper over the flask's top. Next, 5% w/v β-CD was added and stirred for 1 h. In case of formulations without β-CD, this stage was skipped. 2% w/v chitosan was then added and the reaction was stirred for 2 h at 50 °C. All films were obtained by pouring 9 mL portions of the film forming solutions into Teflon Petri dishes (9 cm in diameter). All films spontaneously dried at 23 °C overnight in a chemical hood at RH of 65 ± 2%. The prepared films were stored at −20 °C until their application in the experiments. Acid-base titrations were used to determine PA content in all of the prepared films. Inspected film samples were tested for PA contents in triplicate by extracting them for 2 h in 30 mL DDW at rt. Film samples were chosen from all areas of the inspected films (center areas as well as side areas of the films). Acid-base titrations were then performed with sodium hydroxide (0.1 M) as the titrant and a 1% w/v phenolphthalein solution in alcohol as a pH indicator. All titrations were performed in triplicates per each individual inspected film sample.

### 2.3. Film characterization

#### 2.3.1. FTIR

FTIR spectra of the prepared films were recorded between 400 and 4000 $cm^{-1}$ with 100 scans averaged at a 4 $cm^{-1}$ resolution (Bruker Tensor 27 FTIR Spectrometer).

#### 2.3.2. UV-vis

UV absorption measurements of the prepared films were recorded between 200 and 800 nm on a Shimadzu 1800 UV/Vis Spectrophotometer.

#### 2.3.3. Mechanical properties

Tensile stress (TS), percent elongation at break (PE) and Young's modulus (YM) were determined using an Instron 3345 instrument with an Instron force transducer load cell (Norwood, MA, USA). Tests were performed at a speed of 1 $mms^{-1}$. TS was expressed in MPa and was calculated by dividing the maximum load N by the cross-sectional area $m^2$. PE was calculated by dividing the extension at the moment of rupture by the initial gauge length of the samples and multiplying by 100. YM was expressed in MPa and was determined by the ratio of the stress along an axis over the strain along that axis in the range of stress. All measurements were performed in triplicate for each film type.

#### 2.3.4. Moisture content

For moisture content analysis each film type was weighed in triplicate ($m_w$) with an analytic scale (±0.0001 g) and then dried in an air-circulating oven at 105 °C for 24 h according to ASTM (ASTM, 2009). Films were then reweighed ($m_0$) to determine their moisture content according to: $\%MC = ((m_w - m_0)/m_w) \times 100$.

### 2.4. Antimicrobial activity

The films' antimicrobial activity against stored wheat grains' microflora was inspected by exposing the grains (at moisture

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