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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

content of 12%) to various treatments. 5 g of grains were added to sterile 500 mL beakers. Different additives were then inserted and sealed with gauze strips for 30 days at room temperature with a relative humidity of 65–70% inside the beakers. The examined treatments were: a) control (no additives), (b) PA by direct addition (100 μL or 250 μL, beakers were shaken to assure uniform dispersion), (c) films without β-CD (2 or 5 g, surrounding the grains from all sides as well as top and bottom), (d) films containing β-CD (2 or 5 g, surrounding the grains from all sides as well as top and bottom), (e) films that were grinded to powder (5 g, dispersed evenly between the grains), (f) calcium propionate (250 mg, dispersed evenly between the grains). All of the treatments above were tested in six replications, in four repeating identical experiments. Following the 30-day incubation period, beakers were opened and the grains were taken for infestation evaluation using the direct plating method. A grain external wash (surface sterilization by 2 min in a 2% sodium hypochlorite solution, followed by 2 min rinsing in DDW) was performed when mentioned. Grains were then placed in Petri dishes (ten grains per plate) with potato dextrose agar containing 0.005% chloramphenicol. The number of infested grains was counted daily for 10 days. The statistical test for homogeneity was done on the data from the four repeated experiments and there were no statistical differences, thus the data were combined.

2.5. Statistical analysis

Microsoft Office Excel spreadsheets were used to calculate means, standard deviations and 95% t confidence intervals. The statistical analyses were carried out using the JMP statistical software program, version 7 (SAS Institute Inc., Cary, NC, USA) including a one-way variance analysis (ANOVA) followed by the Tukey-Kramer honestly significant difference (HSD) post-hoc test. Results marked with different letters are significantly different at $P \leq 0.05$.

3. Results and discussion

3.1. Film formulation, characterization and optimization for maximum efficiency

Chitosan and carboxymethyl cellulose (CMC) were examined to serve as carrier matrices for propionic acid (PA) due to past successful postharvest treatment with food products (Petriccione et al., 2015; Ngamakeue and Chitprasert, 2016; Chen et al., 2014; Ziani et al., 2010). β-Cyclodextrin (β-CD) was incorporated into some of the formulations as an encapsulation agent as was shown before (Poverenov et al., 2013; Rutenberg et al., 2016a), and by doing so to increase the polymer matrices' capacity and retard the active agent's natural release rate. This was possible due to the formation of an inclusion complex between β-CD and PA (Rutenberg et al., 2016b). The general notion is that exposing certain PA containing films to grains during their postharvest stage, may allow a controlled and subdued diffusion of PA from the film matrices, thus circumventing the acid's natural evaporative trait that is predominant when used in its natural form. CMC-based films were prepared successfully and appeared homogeneous and smooth. This characteristic is important since it signifies that a homogeneous PA release can be achieved from all areas of the film, ensuring a homogeneous dispersion of the fungistat from all directions. Films made of CMC alone presented their natural transparent quality and were recorded with the most light transmittance via UV-vis measurements. Adding PA to this formulation has led to a small loss in light transmittance. β-CD's involvement in CMC-based film formulations had the most dominant effect, as almost all of the light transmittance was lost in the latter formulation with the films appearing opaquer. Light transmittance in the films can prove as a contributing factor in mold growth. Postharvest grains that are surrounded by a material with high light penetration are susceptible to increased fungal inoculation. This is also an indication of how strongly different molecules can exert a physical change on their surrounding CMC polymeric architecture. PA does not alter CMC's natural polymer characteristic in a drastic measure, whereas the combination of both PA and β-CD in a CMC-based film formulation leads to a more prominent change in the matrix architecture (Fig. 1, Fig. S1). Prepared films were spontaneously dried in petri dishes, causing their surrounding outlines to fold and attach to the dishes' plastic rims. For that reason, the films' outlines appear less than homogeneous in regards to their central regions. All spectral investigations were performed at the films' centers and represent their well-defined homogeneity.

Chitosan-based film formulations presented a similar trend and were obtained largely as smooth, transparent and homogeneous (excluding formulations with β-CD). These films showed a good structural integrity with a typical chitosan-like brown coloration. A simple chitosan formulation displayed lower light transmittance than that of its CMC counterpart. Addition of PA caused an increase in light transmittance, indicating a change in the polymer chains' nature. This observation is in accordance with further FTIR measurements and antifungal test results.

Chitosan and CMC-based films were then studied for their moisture content (Table 1) as possible moisture contributors to the grains other than the surrounding atmospheric conditions. Addition of PA to the chitosan-based film formulation has increased its moisture content due to the fact that PA increases the general hydrophilic nature of the polymer matrix. Addition of both PA and β-CD to the film formulation has dropped the moisture content significantly, allowing for a better formulation that maintains dryer conditions further inhibiting fungal growth. The PA/β-CD system is known to include water molecules trapped in an inclusion complex structure at the expense of the polymer matrix' moisture content. CMC-based films were found to form a similar trend. PA involvement increases the polymer's moisture content, whereas a PA/β-CD inclusion complex incorporation leads to a reduction in moisture content.

Films were also analyzed for their mechanical properties; tensile stress (TS) - the force required to break a film apart by pulling it in opposite directions, percent elongation at break (PE) - the films' elasticity that denotes their degree of elastic deformation before breaking, and Young's modulus (YM) - which relates to the amount of force required for a specimen's deformation. A high YM value indicates a material's tendency towards rigidness. When considering these films as part of a large scaled postharvest storage unit, grains of massive weights have to be accounted for. These can crush the films or distort them, leading to premature PA release. The films' different formulations' mechanical properties hence must be accounted for. Results from the mechanical tests have revealed that values for either chitosan or CMC-based films are not altered significantly when adding PA (Table 1), in accordance with our previous UV-vis observations. Upon including β-CD in the films' formulation, their TS, PE and YM values were all reduced, in accordance with previously published work (Rutenberg et al., 2016a). This is due to β-CD's disruption in both polymer chain continuity, and chain-to-chain interactions. Overall, the chitosan-based formulations were found to be less rigid than their CMC-based analogues, in addition to demonstrating better elastic qualities. Despite their overall detrimental effect to the films' mechanical properties, the addition of β-CD to the films' formulation is still favorable with their increased fungistat capacity that allows for their improved mold inhibition.

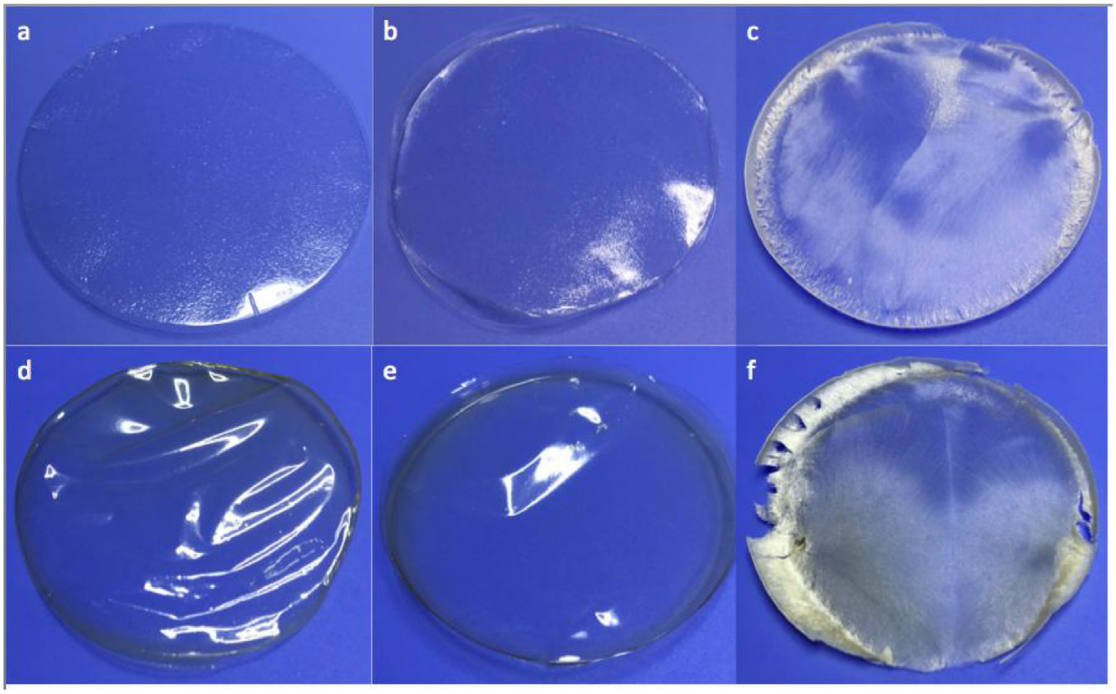

Fig. 1. Prepared film formulations' general appearance. a) 2% CMC; b) 2% CMC, 15% PA; c) 2% CMC, 15% PA, 5% β-CD; d) 2% chitosan; e) 2% chitosan, 15% PA; f) 2% chitosan, 15% PA, 5% β-CD.

Table 1
Mechanical properties and moisture content for the prepared film-based delivery systems. Tensile stress (TS) and young's modulus (YM) are expressed at MPa units, percent elongation (PE) and moisture content (MC) at %. Reagents are presented in % w/v. Values represent means of three replications and 95% t-based confidence intervals, followed by ± as standard deviations. Letters represent comparisons of the mechanical values. Values followed by different letters are significantly different according to Tukey–Kramer HSD test at $p \leq 0.05$.

Formulation	TS	PE	YM	MC
2% CMC	59.5 ± 6.3^{a}	7.7 ± 0.7^{ab}	1701.7 ± 352.1^{a}	21.2 ± 0.3^{a}
2% CMC, 15% PA	61.1 ± 5.9^{a}	12.6 ± 4.7^{a}	1765.8 ± 191.7^{a}	21.7 ± 0.2^{a}
2% CMC, 15% PA, 5% β-CD	4.9 ± 2.2^{b}	4.0 ± 0.8^{b}	261.0 ± 40.6^{b}	12.7 ± 2.4^{c}
2% Chitosan	37.7 ± 17.2^{a}	15.8 ± 0.3^{a}	1058.5 ± 372.7^{a}	15.1 ± 0.7^{bc}
2% Chitosan, 15% PA	32.8 ± 4.1^{a}	8.7 ± 3.8^{ab}	883.8 ± 166.5^{ab}	17.8 ± 0.1^{b}
2% Chitosan, 15% PA, 5% β-CD	5.8 ± 1.6^{a}	1.9 ± 1.3^{b}	453.4 ± 97.3^{b}	12.5 ± 0.8^{c}

3.2. Film optimization, exposure and subsequent safe grain inoculation

PA, the most commonly used fungistat, is applied to grains as a pure acid. Several disadvantages arise from it being applied as a pure liquid, *e.g.*, its corrosive nature towards metal containers, and its necessity to be applied uniformly and in adequate amount in relation to the substrate's water content. There is a need to develop a strategy by which PA will be applied not as a liquid but in another form, one that will display safer and more environmentally friendly traits. Various delivery formulations that included encapsulated PA were tested for their ability to inhibit mold development in wheat grains in a safer film-based approach (Fig. 2). Grains in this specific experiment did not undergo surface sterilization in order to initially test both internal and external microflora. The inspected film formulations relied on chitosan and CMC matrices as the delivery systems' biodegradable platforms. An identical PA concentration was used for all film formulations (15%), and its incorporation into the polymers assisted in its later diffusion to the wheat grains. Formulations with PA encapsulation were obtained by combining it with β-CD (5%) in solution prior to the polymers' introduction. β-CD is known to increase PA concentration and slow down its natural release rate in a polymer-based film formulation (Rutenberg et al., 2016a). It was found that chitosan-based films did not show a significant antifungal effect. Their effectiveness was barely a day's worth more than the control treatment, and β-CD's addition did not facilitate in increased antifungal results. CMC-based films with PA's antimicrobial effects were moderate. However, when β-CD was involved, a remarkable mold inhibition activity was demonstrated. The version without β-CD reached an 80% inoculation after 4 days, while parallel films with β-CD showed a full inoculation after 10 days, more than doubling PA's effective time frame. Control grains with no treatment were used as a reference point and showed 100% inoculation within 48 h.

In a follow-up experiment, grains that underwent surface sterilization were also tested against the same formulations in order to test their effects on the internal microflora alone. In this case as well, CMC-based films with β-CD managed to better inhibit fungal development in grains than films without β-CD, proving the former formulation's effectiveness against both internal and external microflora (data not shown).

Various formulations' residual PA amounts left after their exposure to the grains have ended were measured and compared to their respective initial amounts. It was found that CMC-based films

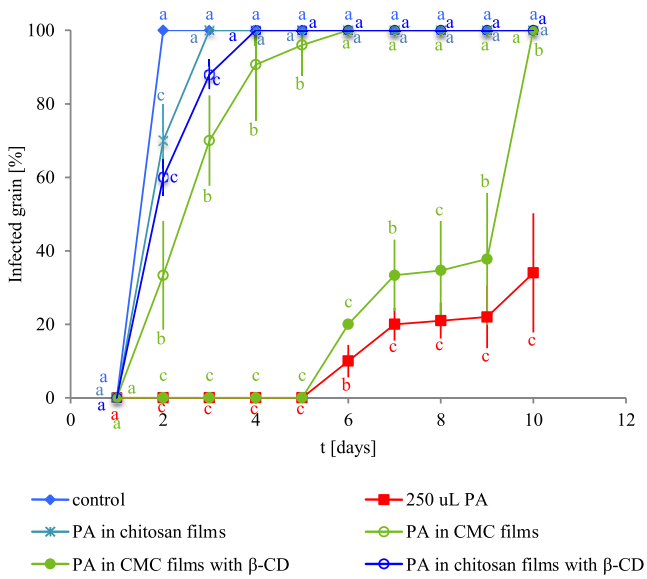

Fig. 2. Various PA formulations and their ability to inhibit mold development in wheat grains. Values represent means of six replications, in four repeating identical experiments each, accompanied by an error representing the standard deviation for these replications and repeated experiments. Values followed by different letters are significantly different according to Tukey–Kramer HSD test at $p \leq 0.05$.

usually lost ~90% of their initial PA content, while chitosan-based analogues only lost ~10% in comparison. This measurement explains the chitosan formulations' poor antimicrobial activity. Chitosan-based films were therefore concluded to not demonstrate sufficient encapsulated PA active release for a satisfying fungal growth delay in wheat grains. The reason for such a drastic difference in PA release between the two biopolymer-based formulations is based on an ammonium carboxylate salt formation that takes place in chitosan's film forming solution, and blocks PA's spontaneous release from it (Kinbara et al., 1996a,b). Collected FTIR scans have confirmed this assumption, as Fig. S2 shows there is no difference in CMC-based films' spectrum when PA is added to the formulation, hence the acid is embedded inside the polymer without being modified. However, a similar comparison of chitosan-based films with and without PA reveals the difference that lays beneath the primary amine's and ammonium carboxylate salt's overlapping band frequencies. Chitosan's 1639 cm^{-1} peak is slightly shifted to 1641 cm^{-1} when PA is involved in the formulation, suggesting a change in the polymer's nitrogen functional groups. The peak's nature and intensity had also changed as it underwent a transformation to an ammonium carboxylate salt. Furthermore, PA's carboxyl characteristics have all disappeared once introduced into the films.

This conclusion, together with CMC-based films' greater PA diffusion at the end of their exposure time to grains, can lead to their applicative use as recyclable materials. Used films can be separated from agricultural products and re-loaded with PA to renew their potency for multiple treatments, making this a non-disposable approach, increasing its efficiency. In addition, the films' polymeric structure can be related to their activity. PA's chemical modification due to an undesired chemical reaction with chitosan's amine groups leads to the formed film to be deprived almost entirely of any fungistatic activity. CMC however, is comprised of different functional groups that allow it not to covalently bond to PA, permitting the acid's controlled diffusion through the polymeric network.

Once an optimal formulation for encapsulating PA was established (CMC-based films with β-CD), its required dosage for an effective fungal growth inhibition was examined (Fig. 3). 2 g and 5 g of CMC-based films with encapsulated PA were tested, as well as their equivalent neat PA amounts (100 and 250 μL, respectively).

While control grains were entirely inoculated within 3 days, complete grain inoculation by 2 g of encapsulated PA active films was reached after 5 days, showing this amount is not sufficient for an effective fungal growth inhibition. Grains that were exposed to a 5 g dose of encapsulated PA active films showed a similar inoculation rate as the 100 μL PA treatment. However, a 100% inoculation was recorded one day before that of grains that were exposed to 100 μL PA. Based on these results and taking into consideration the advantage of using encapsulated PA vs. its liquid form, more effort should be devoted to better develop the "encapsulated form". This novel approach yields a significant advantage in its safer application.

The effectiveness of a powder formulation versus a film formulation was also examined (Fig. 4). The aim of this experiment was to try another form of PA delivery with an increased surface area and different dispersion possibilities. Powder was obtained by shredding prepared films prior to grain introduction. The powder formulation showed poor fungal inhibition abilities, most similar to the control grains, as they lost close to 90% of their initial PA content due to the shredding process. Films with encapsulated PA in this experiment exhibited a significant fungal inhibition activity. A larger surface area is still believed to potentially serve as a better formulation, yet its preparation process should not hinder PA's encapsulation or untimely release. For applicative uses, biodegradable beads and smaller films are considered as suitable candidates for larger surface area modifications.

Additional treatments with calcium propionate were examined as a solid form of delivery for PA. Calcium propionate yielded poor inhibitive results, which resembled the control treatments. This is thought to stem from its poor volatility, despite of its better dispersion abilities in the bulk grains.

As a whole, PA's liquid form was found to be preferable in inhibitive results only. Its direct addition is widely used today in the agricultural industry yet still suffers from major drawbacks, mainly due to uncontrollable expedited evaporation, lack of an ability to be

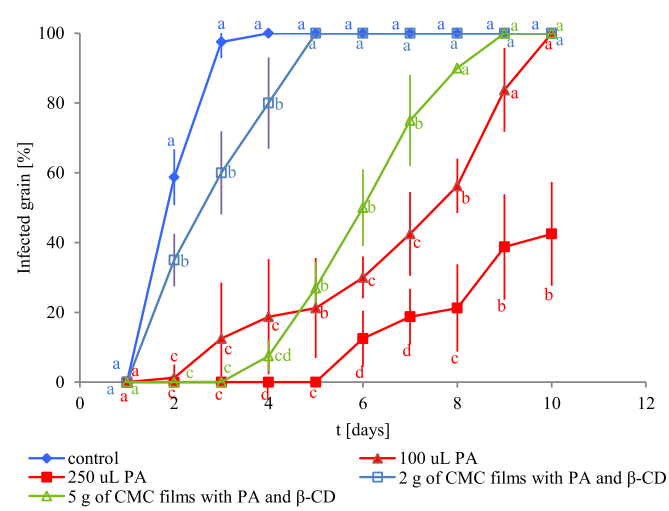

Fig. 3. Dosage effect of encapsulated PA films versus neat PA treatments and their ability to inhibit mold development in wheat grains. Values represent means of six replications, in four repeating identical experiments each, accompanied by an error representing the standard deviation for these replications and repeated experiments. Values followed by different letters are significantly different according to Tukey–Kramer HSD test at $p \leq 0.05$.

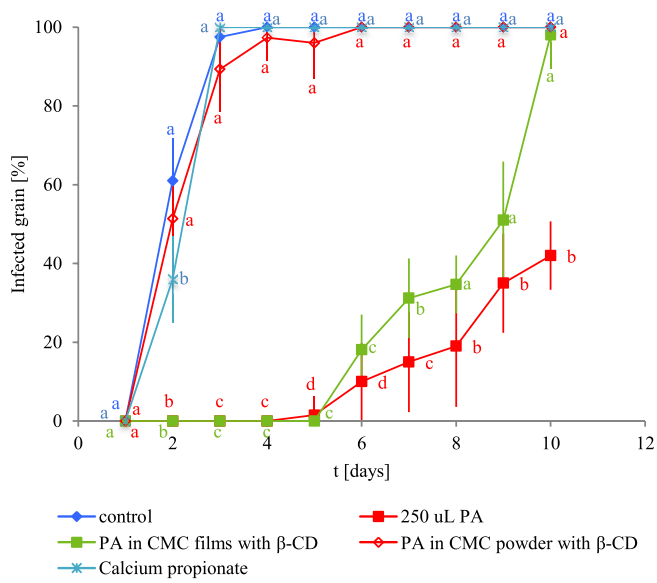

Fig. 4. Effect of film formulation versus powder formulation and their ability to inhibit mold development in wheat grains. Values represent means of six replications, in four repeating identical experiments each, accompanied by an error representing the standard deviation for these replications and repeated experiments. Values followed by different letters are significantly different according to Tukey–Kramer HSD test at $p \leq 0.05$.

distributed in a homogeneous manner among dry food products and major safety concerns when dealing with the acid directly. In this study, encapsulated PA was added to the grains by a solid carrier medium that delivered it selectively as a diffused gas. This formulation allowed controlling PA's vapor pressure in a closed environment by adjusting its mass and diffusion rate. The diffused gas' release mechanism relies on its inclusion complex structure. While encapsulated, its release rate is retarded due to a combination of hydrophobic and hydrogen-bond interactions with its β-CD host (Rutenberg et al., 2016b). It then experiences attractive forces with the CMC polymer's carboxyl functional groups that further inhibit it from being released spontaneously. The sum of these interactions is the chemical core for explaining the fungistat's controlled and unnatural release rate, also increasing its safe use. Further fine-tuning PA's encapsulating systems and governing their chemical and physical interactions may achieve even further diffusion rate modifications.

4. Conclusions

A CMC-based formulation encapsulating PA, composed of natural biodegradable materials, was shown to successfully inhibit fungal growth in wheat grains as part of a recyclable approach. This approach allows a non-direct antimicrobial agent application in agricultural products, with safer and controlled release benefits. PA's capacity and effective release were found to be enhanced with the aid of β-CD as a host agent. When encapsulated, PA has shown to be present in greater amounts in the examined wheat grains, as it inhibited both internal and external microflora development and for longer durations. Film formulation was found to be the most successful method of delivery for the investigated encapsulation system, although future studies should inspect formulations with greater surface areas. This laboratory-scale experiment has shown encapsulated PA's potential in the improvement of an antimicrobial agent to be used in stored dry foods, with an improved efficiency expressed in better safety, applicability and reduced financial losses.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cropro.2017.09.005.

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