



# Physicochemical, antioxidant and antibacterial properties of dispersion made from tapioca and gelatinized tapioca starch incorporated with carvacrol



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## ABSTRACT

The present study investigated the properties of tapioca and gelatinized tapioca starch dispersion and film enriched with carvacrol as antioxidant and antimicrobial agent. The gelatinized tapioca had higher pH and conductivity and similar zeta-potentials and particle sizes than tapioca. Carvacrol decreased the pH, conductivity and particle size and increased the zeta-potential of the dispersions. Tapioca recorded greater viscosity than gelatinized tapioca. The viscosity of the tapioca and gelatinized tapioca dispersions decreased as the rotational speed of the spindle increased, indicating that the dispersions were non-Newtonian shear-thinning fluids. Carvacrol caused a significant increase in the viscosity tapioca starch. Tapioca starch film had greater tensile strength, elongation at break, swelling and water vapor permeability than the gelatinized tapioca starch. Carvacrol significantly decreased the tensile strength and swelling and significantly increased the flexibility, solubility and water vapor permeability of the tapioca film. Scanning electron microscopy revealed the effect of carvacrol on the film microstructure which caused discontinuity in the film matrix. Carvacrol considerably increased antioxidant and antibacterial activity of the starch dispersions. *Pseudomonas aeruginosa* was the most resistant bacteria. The improved antioxidant and antimicrobial activity of the dispersions indicate that such products can be used as bioactive film.

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## 1. Introduction

In recent century the abundant use of synthetic antibiotics has resulted in the appearance of a number of drug resistant bacteria, fungi, yeast and parasite in food yields. To overcome the increasing resistance of pathogenic microbes, more effective and safe antimicrobial agents must be developed. The antimicrobials can be directly added into the product formulation, coated on its surface or incorporated into the packaging materials ([http://www.ncbi.nlm.nih.gov/pubmed?term=Arora%20A%5BAuthor%5D&cauthor=true&cauthor\\_uid=20492194](http://www.ncbi.nlm.nih.gov/pubmed?term=Arora%20A%5BAuthor%5D&cauthor=true&cauthor_uid=20492194), Arora & Padua, 2010). Incorporation of antimicrobials into foodstuff results in an immediate reduction of microorganisms, while the antimicrobial active packaging can maintain their activities for a long period of time (Lucera, Costa, Conte, & Del Nobile, 2012). Biopolymers (starch,

cellulose, chitosan, casein, whey protein, collagen, egg white, soybean protein, zein, gelatin and wheat gluten) have attracted considerable attention as a potential replacement for packaging materials against synthetic polymer.

Biodegradable polymers such as starch have been studied as a potential active film because of their good physical, mechanical, and barrier properties. The popularity of starch relates to its abundance, renewability and relatively low cost; however, starch alone has limitations related to its hydrophilic nature and lack of antioxidant and antimicrobial properties (Shi et al., 2007; Souza, Goto, Mainardi, Coelho, & Tadini, 2013). Enriching of starch with natural antioxidant and/or antimicrobial substances will extend the functional properties of it and provide a bioactive biomaterial (Perazzo et al., 2014; Souza, Ditchfield, & Tadini, 2010). There is growing interest in using plant extracts as natural sources of antioxidant/antibacterial compounds in the formulation of starch. In this context, plant essential oils and their main components are gaining a wide interest in the health industry for their potentials as antioxidant and antimicrobial agents (Gomez-Estaca, Lopez-de-

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Dicastillo, Hernandez-Munoz, Catala, & Gavara, 2014; Solórzano-Santos and Miranda-Novales, 2012). One of the bioactive agents with potent antioxidant and antimicrobial activity is carvacrol. Carvacrol (5-isopropyl-2-methyl phenol) is a phenolic monoterpenes in essential oils derived from genera *Origanum*, *Thymus*, *Coridothymus*, *Thymbra*, *Satureja*, *Lippia* and *Zataria*. It has been used for many generations as a food preservative. The antioxidant, antibacterial, antifungal and antiparasitic activities of carvacrol have been confirmed (Can Baser, 2008). Carvacrol is one of the useful plant materials with potent antioxidant (20–30 µg/ml depending on oxidants) and antibacterial (20–55 µg/ml depending on bacteria) activities (Kavoosi & Rabiei, 2015).

Few studies have been done on the rheological properties and antioxidant/antibacterial activity of carvacrol dispersed in tapioca and gelatinized tapioca starch dispersion and film. In the present study, tapioca and gelatinized tapioca starch dispersions were prepared and carvacrol, as a potent antioxidant and antimicrobial agent, was dispersed into them. The zeta-potential, particle size, viscosity, antioxidant, and antibacterial activities of the dispersions were further investigated. The tensile strength, elastic modulus, elongation at break, solubility, water barrier property, and swelling of the film were also investigated. The study was conducted in two steps. The properties of the tapioca and gelatinized tapioca starch dispersions and film were determined and then the effects of carvacrol on their properties were evaluated.

## 2. Materials and methods

### 2.1. Chemical compounds studied in this article

Starch (CID: 24846132); carvacrol (CID: 10364); glycerol (CID: 753); citric acid (CID: 311); 1-diphenyl-2-picryl-hydrazyl (DPPH) (CID: 2735032); 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (CID: 5360881); potassium persulfate (CID: 24412); silica gel (24261); methanol (CID: 887); agar (CID:122172885); magnesium nitrate (CID: 25212).

### 2.2. Preparation of dispersions

One gram of Tapioca starch or gelatinized (modified) tapioca starch (Poya Kabak manufacturing and trading company, Tehran, Iran) was dissolved in 80 ml of distilled water under continuous stirring at 70 °C until a homogenous solution was obtained. The homogeneous solution was sonicated using an ultrasonic (Bandlin; Germany) at 140 W for 5 min at 30 °C. To preparation of starch/carvacrol dispersions, carvacrol (100 mg/g based on the weight of starch powder) was added to the solution and mixed under mechanical stirring at 800 RPM for 12 h at 40 °C. Glycerol (30% w/w) was added as a plasticizer to the dispersions and mechanically stirred at 800 RPM at 40 °C citric acid (10% w/w) was added as a cross-linker to the dispersion and mixed under mechanical stirring at 800 RPM at 40 °C. Distilled water was added to a final volume of 100 ml under mechanical stirring at 800 RPM at 40 °C. The final dispersions were stored at 4 °C until testing. The dispersion was stable for at least 100 days as determine by visual observation of testing phase separation (Menzel et al., 2013).

### 2.3. Conductivity and pH of starch dispersion

A Mettler-Toledo instrument (Cleantech; Switzerland) was used to measure the pH and conductivity of the dispersions.

### 2.4. Zeta-potential of starch dispersion

The dispersions were diluted to a final concentration of 50 µg/ml

using deionized water. The electrophoretic mobility of the particles in dispersion was determined by phase analysis light scattering (PALS) using a 90 Plus zeta-sizer (Brookhaven; USA) according to manufacturer instructions. Bi-PALS zeta potential analyzer software provided the average of electrophoretic mobility and measured the zeta potential using the Smoluchowski model.

### 2.5. Hydrodynamic particle size of starch dispersion

The equilibrated dispersions were diluted to a final concentration of 50 µg/ml with deionized water. The effective hydrodynamic diameter of the nanoparticles was determined using the principle of dynamic light scattering (DLS) using a 90 Plus particle size analyzer (Brookhaven; USA) according to manufacturer instructions. Bi-9000 particle sizing software provided the average effective hydrodynamic diameter of particles.

### 2.6. Apparent viscosity of starch dispersion

The dispersion was decanted into a glass cylinder (80 × 70 mm) and left on an anti-vibration bench having a flat surface for 10 min to reach equilibrium. The apparent viscosity (mPa.s) of the dispersion was measured using a Brookfield viscometer (DVII-pro; USA) at a spindle (no. 2) rotational speed of 1, 2, 5, and 10 s<sup>-1</sup> according to manufacturer instructions. The software provided the apparent viscosity of the dispersion directly (Almeida et al., 2013).

### 2.7. Film preparation

The dispersion was poured onto a flat level polystyrene petri dish (Farazbin; Iran) for film casting. Once set, the petri dish was held overnight for 12 h in a vacuum oven (Fan Azma Gostar; Iran) at 50 °C for the dispersion to yield a film of uniform thickness. The film was then cooled to ambient temperature before being peeled off the petri dishes. For further testing, the film samples were equilibrated in a desiccator at 60% ± 5% relative humidity over a saturated solution of magnesium nitrate for 3 d. The film samples were then stored in plastic bags at 4 °C. Thickness of the film was measured with a micrometer (Starrett; Uk) and the average of 150 ± 5 µm was used for tensile testing and water vapor permeability determination (Reddy & Yang, 2010).

### 2.8. Mechanical behavior of the film

The tensile strength, elongation at break, and elastic modulus were determined according to ASTM method D 882-00 (ASTM, 2002) with an Instron testing machine (Santam; Iran) equipped with a tension cell of 100N. The film was cut to 60 × 10 mm. The area of film used for each experiment was 60 × 10 mm; however, 20 mm of the film fell within the jaws, so the initial length of the film was taken as 40 mm. The thickness of the film was measured at different points with a micrometer and the average taken. The initial cross-sectional area of the film cut was 10 mm × average thickness in mm. The film cut was conditioned at 27 °C and 60% ± 5% relative humidity by placing it in a desiccator over a saturated solution of magnesium nitrate for 2 d. Tensile strength testing was then performed by stretching the film cut at a speed of 50 mm/min. The software of testing machine directly provided the tensile strength, elongation at break, and elastic modulus of the film. All the mechanical tests were carried out at room temperature (Menzel et al., 2013).

### 2.9. Solubility of the film

The film was cut into a 20 mm radius disc, dried in a vacuum

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