

Effect of Carnosine on Physico-Chemical and Antioxidant Activity Properties of FSG-CaCO₃ Composite Films

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Abstract. For the sake of developing fish skin gelatin and CaCO₃ composite films with antioxidant activity, different concentration of carnosine was added to it. In addition, the mechanical properties, optical properties, surface topography, anti-microbial activity, antioxidant activity and preservation effect of carnosine-FSG-CaCO₃ were also investigated and analyzed. The results indicated that the incorporation of carnosine caused interactions between gelatin and ingredients in carnosine, and the films showed increased thickness and elongation. After the accretion of carnosine, the films showed darker appearance, and decreased tensile strength, water solubility and water vapor permeability. Meanwhile, the inhibition to food oxidation and preservation effect enhanced, and the surfaces became rougher by AFM with the increase of concentration of carnosine. In summary, carnosine-FSG-CaCO₃ composite films incorporated with carnosine presented application capacity in food packing and preservation.

Introduction

Biological polymers with high biodegradability and good biocompatibility, has been widely used in food, cosmetic and pharmaceutical industries [1]. Different kinds of food additive could be added in the biological macromolecule polymer, such as: antibacterial, antioxidant, antifungal agent, coloring matter and other nutrients, to improve food quality and prolong the shelf life of food [2]. Biological polymers, such as polysaccharides and lipids, compared to different sources of protein, showed better film-forming ability, high water binding capacity and emulsifying properties [3]. Fish Skin Gelatin (FSG) from Fish Protein isolated (FPI), which has good holding water, membranous and emulsification properties, has been used to the development of new type of edible films [4].

Calcium carbonate is a stable and safe natural substance, mainly derived from marine organisms, rocks and shell, etc. CaCO₃ has been found to behave as a physical barrier to protect the body in the natural environment from external mechanisms and microbial damage [5]. In general, it is used to make biodegradable natural source of filling materials. In addition, after the importance of calcium in heart, nervous system, muscles and bones have been proved, CaCO₃ is considered as a kind of food fortifier [6].

Recent years, CaCO_3 has been widely used in microcapsules, tablets [5, 7] [8] and composite films.

In this paper, different concentration gradients of carnosine were added to FSG- CaCO_3 (CaCO_3 0.5% w/v or 10% to FSG) composite films to improve antioxidant activity and study the characterization of the edible films. This characterization included mechanical behavior, water barrier ability, optical properties, microstructure and surface morphology. Additionally, the antioxidant capacity of films applied to pork packaging to protect against lipid oxidation was determined.

Material and Methods

Materials

Commercial fish skin gelatin (FSG) from tilapia (~ 240 blooms) and carnosine was purchased from Lapi Gelatine S.p.A (Empoli, Italy); Glycerol and Calcium carbonate was purchased from Sinopharm Group Chemical Reagent Co., Ltd. (China) was of analytical grade.

Preparation of the Film-Forming Solution and Casting of the Films

Prior to blending, FSG powder was slowly dissolved in distilled water at 45 °C and stirring for 2 h to obtain the protein concentration of 5% (w/v). Glycerol (40 % w/w to gelatin) was added as mentioned above. The film-forming solution were obtained by adding carnosine in the following content 0.1% w/v, 0.2% w/v and 0.4% w/v. Afterward, the film-forming solution was immediately cast onto polyacrylic plates (11 cm×11 cm) following by the oven-dry treatment for approximately 5h at 30 °C. The prepared films were peeled off and stored in a desiccator with saturated magnesium nitrate ($\text{MgNO}_3 \cdot 6\text{H}_2\text{O}$) for 48 h at room temperature (20 °C) and 53% relative humidity (RH).

Film Thickness

The thickness of film was measured using a digital micrometer (Mitutoyo, Model ID-C112PM, Serial No.00320, Mituyoto Corp., Kawasaki-shi, Japan).

Mechanical Properties

According to GB13022-1991 [9], tensile strength and elongation at break were measured.

Water Solubility

Water solubility of films were tested as followed:

$$WS(\%) = \frac{m_1 - m_2}{m_1} \times 100 \quad (1)$$

m_1 : films weight before dissolving (g); m_2 : films weight after dissolving (g).

Water Vapour Permeability

Water vapour permeability were measured according GB1037-88 [10].

Colour

Total difference in colour was calculated according to Gennadios et al., 1996.

Light Transmittance and Transparency Value

The light transmittance of films was measured at the ultraviolet and visible range (200-800 nm) using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) according to the method of Shiku et al. (2004).

Surface Morphology

The surface of films was studied by Atomic Force Microscopy, using a Nanoscope III. (Digital Instruments, Inc. Santa Barbara, Clifornia).

Measurement of Peroxide Value to Pork Package

Peroxide values (POVs) were calculated.

Total Volatile Bases Nitrogen (TVB-N) Analysis

TVB-N content in pork meat was measured by a stream distillation method, based on the previous report.

Statistical Analysis

SAS (institute inc. cary, NC, USA) was applied to the data statistical analysis.

Results and Discussion

Mechanical Properties Analysis

Table 1 showed the effect of different concentration of carnosine on mechanical properties of FSG-CaCO₃ complex film.

Table 1. Effect of different concentration of carnosine on mechanical properties of FSG-CaCO₃ complex film

Mechanical properties	Samples			
	Control film	0.1% w/v carnosine	0.2% w/v carnosine	0.4% w/v carnosine
Thickness/mm	0.068 ± 0.003 ^d	0.084 ± 0.004 ^c	0.095 ± 0.003 ^b	0.108 ± 0.003 ^a
TS/MPa	21.96 ± 0.104 ^a	20.75 ± 0.198 ^b	18.307 ± 0.254 ^c	16.55 ± 0.401 ^d
EAB/%	5.37 ± 0.960 ^d	9.69 ± 0.281 ^c	21.93 ± 0.511 ^b	31.99 ± 0.552 ^a
WS/%	37.54 ± 2.09 ^a	36.59 ± 1.31 ^a	35.71 ± 23.2 ^a	34.76 ± 1.91 ^a
WVP/(G/(Pa s m))	5.37 × 10 ⁻⁹ ±	4.16 × 10 ⁻⁹ ±	4.39 × 10 ⁻⁹ ±	3.08 × 10 ⁻⁹ ±
	3.47 × 10 ^{-10a}	3.07 × 10 ^{-10a}	9.88 × 10 ^{-9a}	1.39 × 10 ^{-9a}

Values are given as mean ± SD (n = 3)

Different lowercase letters in the same column indicate significant differences (p < 0.05)

Thickness. The variance analysis results showed that every concentration gradient increased in the films had significant influence on thickness (P < 0.05). It is because carnosine addition in FSG network structure induced interaction with FSG and destroyed the order structures of films [11].

Tensile Strength and Elongation at Break. The tensile strength of composite

films decreases, while the elongation at break increased with the increase of concentration of carnosine in films. It perhaps caused by carnosine addition in film matrix increased the discontinuity of FSG network. [11].

Water Solubility and Water Vapour Permeability. Water solubility and water vapour permeability of the films reduced as a result of the increase of the carnosine concentration gradient. It almost caused by the hydrophobic groups in carnosine to reduce the water solubility and water vapour permeability.

Optical Properties Analysis

The effects of different concentration of carnosine on optical parameters of FSG-CaCO₃ complex film were showed in Table 2.

Table 2. Effect of different concentration of carnosine on optical parameters of FSG-CaCO₃ complex film

Index	Parameters	samples			
		Control film	0.1% w/v carnosine	0.2% w/v carnosine	0.4% w/v carnosine
Color	L*	89.80±0.78 ^a	85.08±1.10 ^b	79.50±1.00 ^c	62.79±1.08 ^d
	a*	0.28±0.32 ^d	-0.65±0.27 ^c	0.81±0.58 ^b	9.18±0.82 ^a
	b*	-6.36±0.61 ^d	3.61±4.22 ^c	18.93±1.33 ^b	34.81±1.23 ^a
	ΔE*	2.68±0.23 ^d	12.59±2.41 ^c	28.92±1.09 ^b	51.36±1.27 ^a
Transparency value	T-value	0.22±0.06 ^a	0.56±0.54 ^b	0.65±0.76 ^c	0.74±0.29 ^c

Values are given as mean ±SD (n = 3)

Different lowercase letters in the same column indicate significant differences (p < 0.05)

Color. With the increase of carnosine in film, the color gradually deepened under visual observation. The L *, a *, b * and Δ E * parameters have significant difference compared with controlled ones (P < 0.05), of which, L * lowered significantly, while a *, b * and Δ E * improved significantly.

Light Transmittance and Transparency Value. With the addition of carnosine in films, the transparency were significantly increased (P < 0.05) compared with the control films. The results showed that different concentrations of carnosine acted as a barrier, could increase the transparency and reduce its light transmittance.

Surface Morphology Analysis

Effects of different concentration of carnosine on surface topography of gelatin-calcium lactate complex film were showed in Table 3. Typical AFM images of the surface topography of gelatin-calcium lactate complex film with different concentration of carnosine indicated that the roughness of films increased with the carnosine addition.

Table 3. Effect of different concentration of carnosine on surface topography of gelatin-calcium lactate complex film

Roughness	Samples			
	Control film	0.1% w/v carnosine	0.2% w/v carnosine	0.4% w/v carnosine
Mean roughness (Ra) /nm	25.42±0.32 ^d	38.58±0.86 ^c	55.35±0.89 ^b	72.28±1.90 ^a
RMS roughness (Rq) /nm	26.77±0.41 ^d	39.10±0.93 ^c	57.17±0.92 ^b	78.34±2.04 ^a

Values are given as mean ± SD (n = 3)

Different lowercase letters in the same column indicate significant differences (p < 0.05)

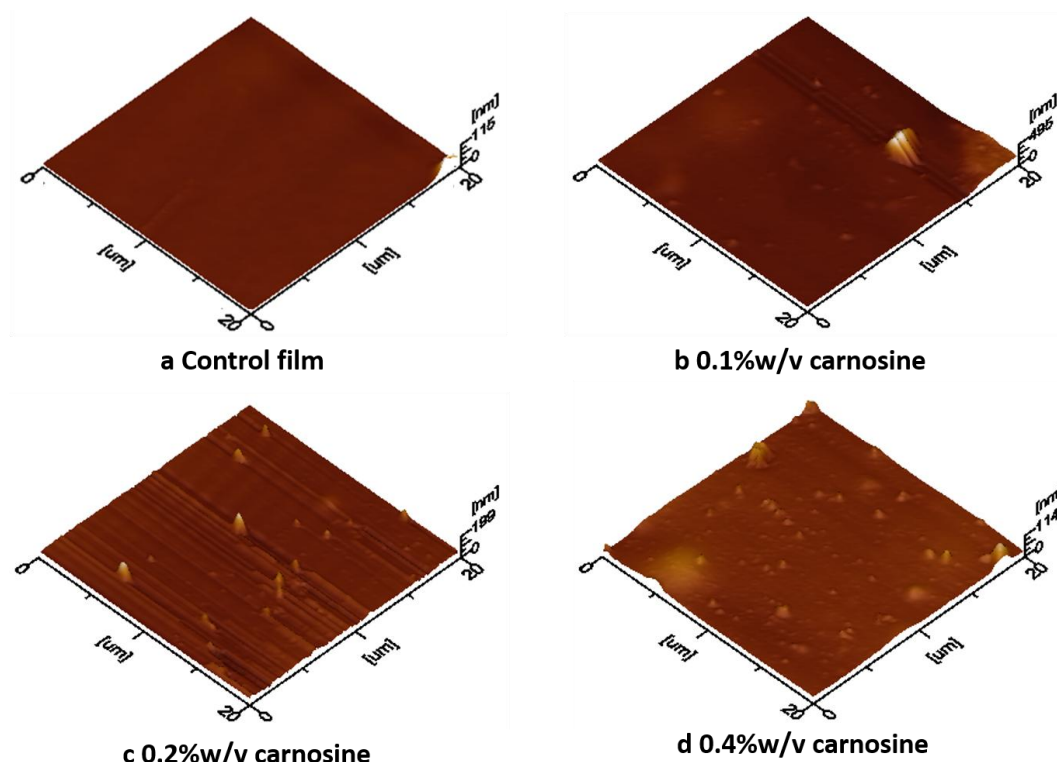


Figure 1. Typical AFM images of the surface topography of gelatin-calcium lactate complex film with different concentration of carnosine

Total Volatile Bases Nitrogen (TVB-N) Analysis

The TVB-N level is an important parameter of the fresh evaluation of meat, because ammonia and amine release during the process of meat corruption. With the extension of time, the TVB-N content is increased as the carnosine concentration addition. The results show that addition carnosine FSG-CaCO₃ composite films can delay the corruption of meat to extend the shelf life of the packaging product.

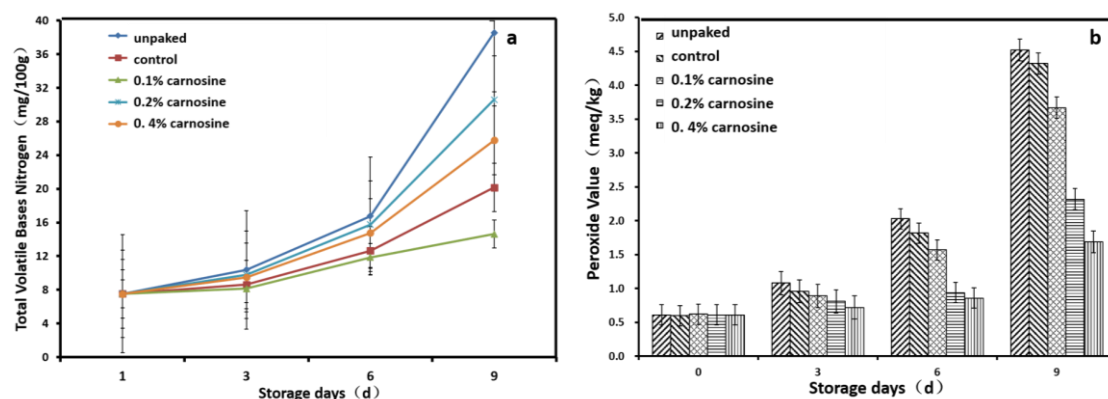


Figure 2. a: Changes of TVB-N of meat packed by gelatin-calcium lactate complex film with different concentration of carnosine in cold-storage; b: The variations of the meat on peroxide value during the storing

Antioxidant Analysis. As shown in Fig. 2-b, the POV of all samples are increased in the process of storage, especially the unpackaged samples. To the controlled films, POV in packaged with films added in carnosine increased less. The results show that FSG-CaCO₃ composite films with carnosine ingredients have the function of the inhibition of lipid oxidation in specimens. FSG-CaCO₃-carnosine composite films obtained antioxidant function could prolong the shelf life of chilled meat.

Conclusions

By adding different concentrations of carnosine FSG-CaCO₃ composite films and propertied analysis, we concluded that: with the incorporation of carnosine in composite films, the thickness of the composite films increased, mechanical strength, tensile strength decreased, while the elongation at break increased, solubility in water and water vapor transmission coefficient reduced. On the optical performance, color appearance is more and more deep. On the surface morphology, the roughness of the films increased. Preservation effect on the volatile base nitrogen content and peroxide value growth were slowed. The above results showed that by addition of carnosine to develop carnosine FSG-CaCO₃ composite films, the inhibition of lipid oxidation and the preservation effect of food were improved to application in food packaging.

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