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Certain Properties of Gelatin-Starch Film Incorporated with Plant Extract (*Kaempferia parviflora* Wall. Ex Baker) and its Application to Meat Product

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ABSTRACT

The objectives of this study was to study the influence of plant extract and starch on gelatin film properties and on rancidity stability of meat products. The effect of the extract was also compared to BHA antioxidant. Physical, functional and antioxidant properties of the films were measured. Development of rancidity parameters of the meat products kept in gelatin pouches was also determined. The extract and starch improved film strength but decreased water vapor resistance. Optical and color properties of film were significantly altered by the extract concentration. The film with extract and BHA exhibited antioxidant activity at 89.83 and 70.36% inhibitions, respectively and significantly decreased hydrolytic rancidity rate of the products. So, plant extract might modify some properties of the gelatin film but it could exhibit antioxidant activity on the gelatin film comparable to synthetic antioxidant. Then it will be a more promising natural additive to preserve meat products in gelatin packaging.

Key words: Antioxidant, BHA, extract, packaging, storage

INTRODUCTION

Biodegradable films made from renewable resources are an important factor in reducing the environment problem of plastic packaging. Studies were carried out with natural film/coatings to preserve food products have been reported. In general, polysaccharides and proteins are widely used for edible coating/film/package making. Protein-based films have good properties to protect products from gas (Jiang *et al*., 2007). Gelatin, hydrolyzed collagen, has been reported as an outer film to protect food from vapor, oxygen and light (Arvanitoyannis, 2002) and is used for commercial edible coating/film including sausage casing from collagen, confectioners and pharmaceutical coating. The gelatin industry primarily generally uses mammalian skins and bones as raw materials. In Thailand, catfish (*Clarias batrachus*) skin is a low market value by-product. Gelatin from fish is in current trend designing biodegradable materials for food packaging to meet socio-cultural needs. Heating collagen in water leads to the conversion of collagen on to soluble gelatin. This can provide a good gel structure at appropriate pretreatment, concentration, pH and temperature. The physical and structural properties of gelatin influenced by the protein molecular weight distribution and amino acid composition which also play functional properties of the film (Gimenez *et al*., 2009). In addition, methods of film forming and plasticizer application have been

reported is an important factor which specifies the film properties. Application of gelatin for food and pharmaceutical barriers is widely accepted but those for active food packaging are still scarce. BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are the most studied synthetic-antioxidants in active food packaging. Nowadays, the use of natural antioxidants instead of synthetic ones is growing. An active package one like film may serve as carriers for antimicrobial and preservative agents in order to maintain their concentration and control release. However, changes induced by antioxidant agents on properties of packaging material such as gelatin should be investigated. The application of natural plant extract to prevent rancidity reaction has been studied in a number of fish products (Gomez-Guillen and Montero, 2007; Selani *et al*., 2011).

Limitations of antioxidant using in foods include participation in complex reactions and high dose usage in a complex food system. The efficiently antioxidant property of *Kaempferia parviflora* Wall. Ex Baker (Krachai-Dum) has been well documented occurring from the flavone compounds (Yenjai *et al*., 2004). *Kaempferia parviflora* Wall. Ex Baker, Thai ginseng, a plant in a family of Zingiberaceae, is very popular for health promotion in Thailand. Krachai-Dum skin is rich in flavonoid contents as compared with another part. The high total flavonoid contents (24.41 mg/100 mL) was also found in Krachai-Dum wine and it effect on Staphylococcus aureus TISTR 746 inhibition was reported (Sirikhansaeng *et al*., 2008). However, the advantage of *Kaempferia parviflora* Wall. Ex Baker extract on active food packaging has not been investigated. Detrimental effects on mechanical and functional properties and antioxidant activity of such edible films may occur when the proper processing conditions are not met. The objectives of this research was to study the influence of plant extract (*Kaempferia parviflora* Wall. Ex Baker) compared with BHA on the mechanical and antioxidant properties of gelatin films and gelatin-starch composite films. The antioxidative ability on a meat product of the extract in a gelatin-starch pouch was also determined.

MATERIALS AND METHODS

This study was carried out at the end of 2011 to the end of 2013 in the Department of Food Technology and Nutrition, Mahasarakham University (Thailand).

Starch and gelatin preparation: Modified starch form cassava was obtained from Thai Food and Chemical Co., LTD. Gelatin was extracted from Catfish skin according to the method of Hoque *et al*. (2010) with slight modification. The skin was pre-treated by soaking in NaOH solution and acetic acid solutions in order to remove non-collagen protein part according to the method of Yang *et al*. (2008). The extract obtained was filtered through filter paper (Whatman®No.4) and concentrated to 5% total solids at 40°C under vacuum pressure. The extracted gelatin (G) was stored at -18°C until used (not longer than 1 month) for film formation.

Plant extract was obtained from 95% ethanol extraction of dried rhizomes (approximately 7% water content) of *Kaempferia parviflora* Wall Ex Baker. The solvent was evaporated under vacuum pressure at 50°C until the 7-8% yield of the extract was achieved.

Film formation: In the film solution preparation, the extracted gelatin (G) and/or 10% starch solution (S) were mixed in the ratio (G: S) of 10:0, 9:1 and 8:2. The 100 g film solution added with glycerol plasticizer (0.2 g/g total solids in film solution) and blended at 1,500 rpm (Polytron mixer) and then heated at 60°C for 30 min in a temperature-controlled water bath. The film solution was cooled down at 25°C and added to extract (1,000, 1,500 and 2,000 ppm) or BHA (100 and 200 ppm) and stirred at 150 rpm for 30 min. Film samples were formed by casting on to a acrylic plate (12×12 cm) and then dried in a hot air oven at 50°C for 15 h (approximately 14% water content of

film). The dried film was placed in a desiccator with 58% relative humidity for 24 h and then manually peeled off. The film obtained was stored at 58% relative humidity until subjected to the analysis of film properties within 24 h. Gelatin film without the extract and BHA was served as a control sample.

Meat product stability: The meat product was prepared from ground pork by using deep fat frying at 200°C. Approximately 100 g of the meat product containing 15-16% water content was packed in a pouch (50×50 mm) which was formed by heat sealing of the starch-gelatin film incorporated with 200 ppm BHA or 2,000 ppm extract and then kept at 25°C for 9 days. The pouch without any antioxidant was served as the control treatment. The water content, Free Fatty Acid (FFA), Peroxide Value (PV) and TBA-value of products were determined.

Film properties: The mechanical properties (tensile strength and extensibility) of film were determined using a texture testing machine (LLOYD Instruments, UK). The film sample (2×5 cm) with the initial grip length of 3 cm was tested. The tensile strength was measured at 30 mm min^{-1} until the film broke. The maximum load and the final extension at break were calculated as tensile strength and extensibility, respectively. Water Vapor Permeability (WVP) was measured following the method of Hoque *et al.* (2010). The WVP expressed as g mm^{-1} sec^{-1}•$mmHg^{-1}$ was calculated as follows:

$$WVP = WVTR.t/S(R_1-R_2)$$

where, WVTR is water vapor transmission rate (vapor flow in unit time through unit area of tested film area), expressed as g mm^{-2} sec^{-1}, t is film thickness (m), S is saturated vapor pressure at test temperature, R_1-R_2 are relative humidity difference (expressed as fraction) across the tested film. The film color was measured by using a Minolta color meter (CR-300, Japan) expressed as Lightness (L*) redness-greenness (a*) and yellowness-blueness (b*). The transparency of film was measured from the transmittance at 600 nm (Shimadzu, Kyoto, Japan) and the transparency value was calculated as follows:

$$\text{Transparency value} = -\log \frac{T_{600}}{x}$$

where, T_{600} is the transmittance with the specimen in the beam at 600 nm, x is film thickness. The film thickness was measured by using a digital micrometer. Five random positions of each film were used for the thickness determination.

Antioxidant activity: The antioxidant property of the films was analyzed by using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Wu *et al.*, 2003), based on the beaching of DPPH radical by antioxidant agents. The absorbance was measured at 520 nm by using a spectrophotometer (Shimadzu, Kyoto, Japan). The antioxidant activity value, expressed as % inhibition was calculated as follows:

$$\text{Inhibition}(\%) = \frac{A-B}{A}\times 100$$

where, A and B are absorbance value of blank and samples, respectively.

Free fatty acid, peroxide value and TBA value: The free fatty acid values of meat ball products were determined according to Coskun and Ondul (2004) with slight modification. The mixture of sample and ethanol-diethyl ether solution was titrated with 0.02% KOH to the end point. Peroxide values were determined using a titration method with $Na_2S_2O_3$. The TBA value was determined according to AOCS (1998) method Cd 1-90).

Statistical analysis: The results were reported as Mean±Standard deviation values. The experimental data was arranged in a Completely Randomized Design (CRD) with three replications. Analysis of variance was performed by ANOVA. Significant difference between experimental means was determined by using the Duncan's multiple range tests ($p<0.05$).

RESULTS AND DISCUSSION

Table 1 shows color parameters and transparency values of the gelatin films. There were significant impact of extract concentration on both film color and transparency. The L* values were decreased with the extract concentration. Higher a* and b* values were shown at films incorporated with the extracts. An increase of a* value (9.46-15.94) exhibited red color of the films, while an increase of transparency value by the extract concentration, indicating lower light transmittance through the film, was clearly shown at 1,500 ppm and 2,000 ppm extract treatments.

The significant effect of extract and BHA concentrations on the mechanical properties of films is shown in Table 2. The investigation found that the 2,000 ppm extract treatment significant increased tensile strength, while the impact of lower extract concentration was not significantly shown. The effect of plant extract on the mechanical properties of gelatin films was in agreement with a report on gelatin films incorporated with herb extracts, namely, cinnamon, clove and star anise (Hoque *et al*., 2011). Similar results have been reported in cases of borage extract (Gomez-Estaca *et al*., 2009a), rosemary extract (Gomez-Estaca *et al*., 2009b) and seaweed extract

Table 1: Effect of extract and BHA on color and transparency of gelatin films

Treatments	Transparency value	L*	a*	b*
Control	1.13±0.06^{a}	97.92±0.10^{d}	0.10±0.42^{a}	0.67±0.42^{a}
BHA 100 (ppm)	1.38±0.04^{b}	97.43±0.85^{d}	0.25±0.07^{a}	1.14±0.98^{ab}
BHA 200 (ppm)	1.54±0.02^{c}	97.33±0.71^{d}	0.23±0.05^{a}	1.12±0.93^{ab}
Extract 1,000 (ppm)	1.49±0.09^{bc}	80.37±1.29^{e}	9.46±0.95^{b}	1.69±0.86^{b}
Extract 1,500 (ppm)	2.09±0.00^{d}	76.51±0.45^{b}	13.47±0.25^{c}	2.69±0.25^{c}
Extract 2,000 (ppm)	2.15±0.00^{d}	74.62±0.83^{a}	15.94±0.83^{d}	2.73±0.46^{c}

^{a,b,c}Superscript letters in each column are significantly different ($p<0.05$)

Table 2: Effect of extract and BHA on mechanical and functional properties of gelatin films

Treatments	Thickness (mm)	Strength (N)	Extensibility (%)	WVP (10^{-9} g mm m^{-2} sec^{-1}•mmHg)
Control	0.09±0.02^{a}	14.24 ±2.89^{a}	295.17±23.170^{a}	1.77±0.60^{a}
BHA 100 (ppm)	0.09±0.09^{a}	10.82±1.97^{a}	366.65±23.080^{a}	6.73±0.04^{bc}
BHA 200 (ppm)	0.08±0.02^{a}	17.86±8.29^{ab}	431.51±124.93^{a}	7.25±1.10^{c}
Extract 1,000 (ppm)	0.10±0.09^{a}	8.63±2.08^{a}	356.26±37.000^{a}	3.51±2.58^{ab}
Extract 1,500 (ppm)	0.10±0.09^{a}	18.96±3.11^{ab}	314.18±5.9400^{a}	3.64±1.14^{ab}
Extract 2,000 (ppm)	0.10±0.01^{a}	21.48±2.39^{bc}	431.20+9.5800^{a}	5.57±0.76^{ab}

^{a,b,c}Different superscript letters in each column are significantly different ($p<0.05$)

Table 3: Effect of starch on mechanical and functional properties of gelatin films incorporated with BHA and extract

Treatments	Thickness (mm)	Strength (N)	Extensibility (%)	WVP (10^{-9} g mm m^{-2} sec^{-1}•mmHg)
Control	0.09±0.06[a]	14.91±0.95[a]	318.39±28.360[a]	1.92±0.51[a]
10% Starch (BHA)	0.11±0.02[a]	30.23±0.47[c]	796.93±215.45[b]	12.28±8.50[b]
10% Starch (Extract)	0.12±0.05[a]	23.97±2.82[bc]	461.68±31.470[a]	11.58±5.62[b]
20% Starch (BHA)	0.11±0.01[a]	15.94±3.60[a]	517.66±148.78[a]	12.59±6.58[b]
20% Starch (Extract)	0.12±0.02[a]	18.68±6.68[ab]	320.63±175.01[a]	11.03±5.31[b]

[a,b,c]Superscript letters in each column are significantly different ($p<0.05$)

(Rattaya *et al.*, 2009). Rattaya *et al.* (2009) reported that the increased mechanical properties of gelatin film possible occurred by the protein-polyphenol interactions as well as other pronounces (Shi and Di, 2000; Hoque *et al.*, 2011). In addition, hydrophilic group in polyphenol substances can enter into hydrophobic region in gelatin protein.

Structure modification of protein network occurred when the plant extract was added, resulting to harder film formation. Considering the tensile strength and extensibility properties, the 2,000 ppm extract treatment seems to provide a good film sample as compared to the 1,000 and 1,500 ppm extract treatments. Increasing of WVP was observed in the films with 100 ppm BHA and 200 ppm. The tensile strength and extensibility of the film with 200 ppm BHA was not different from the control but the water resistance ability was significant lower.

Table 3 shows impact of starch on the properties of gelatin films incorporated with extract or BHA. A significant increase of tensile strength and extensibility values was found when the appropriate starch portion was used, particularly the gelatin to starch ratio of 9:1. However, a greater starch portion did not alter such film properties. Not only starch portion but also plasticizer type or portion affects the mechanical properties. A decrease of tensile strength, depending upon plasticizer type and gelatin portion of saga starch/fish gelatin film, has been reported (Al-Hassan and Norziah, 2012). Using a high concentration of hygroscopic plasticizer caused more polymer change mobility. However, the hygroscopic effect could not be observed in the present work because a lower concentration of plasticizer was applied. In the present work, starch concentration did not significantly affect film thickness but resulting in more than 5-fold increase of WVP. The WVP improvement is therefore important for of development of gelatin film mixed with starch. The starch portion can alter the WVP property of the composite film when it affects to the ratio between crystalline and amorphous zone, polymeric chain mobility and also the protein-polyphenol interactions (hydrophobic-hydrophilic interactions). However, the plasticizer content and type (water and polyols) are important factor as well (Arvanitoyannis and Biliaderis, 1998).

Incorporation of the extract or BHA into the films provided the anti-oxidative films showing approximately 48-90% inhibition values as analyzed by using the DPPH antioxidant assay (Fig. 1). The use of 1,500-2,000 ppm extract was the most efficient. The low antioxidant activity was shown at 1,000 ppm extract and 100 ppm BHA treatments. The 200 ppm BHA concentration used in this present work was in range of food industry limitation. The BHA could exhibit antioxidant activity comparable to 1,000 ppm to 1,500 ppm extract. Using higher BHA concentration should be limited because of its toxicity. This in-vitro test confirmed that artificial and natural antioxidants could release from the gelatin film. As a consequence, a high concentration of plant extract is more interesting in active film development than BHA. The activity of natural

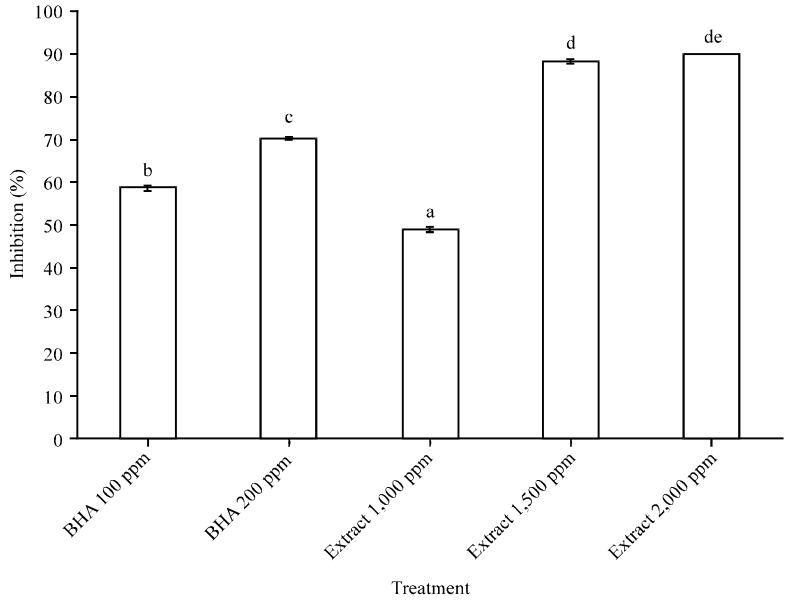

Fig. 1: Effect of extract and BHA on antioxidant activity of gelatin films

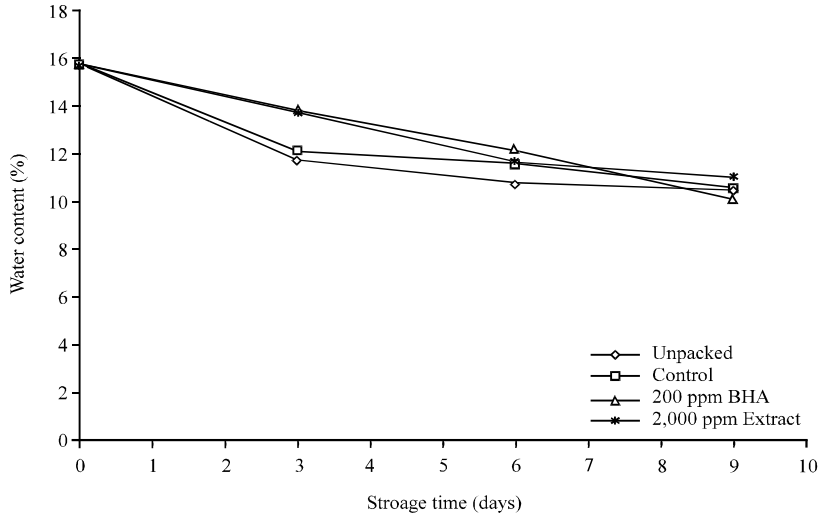

Fig. 2: Evolution of water content of fried meat ball products during storage

antioxidant on gelatin film has been reported. The result was in agreement with the activity of borage extract in gelatin film. Its activity measured by FRAP assay was comparable to α-tocopherol and BHT (Gomez-Estaca *et al*., 2009a). Moreover, borage extract exhibited a rapid reaction within 4 min when compared to BHT. The possible hydrogen bonding with the film matrix might lead to a decrease in the antioxidant activity of BHT (Jongjareonrak *et al*., 2008).

Figure 2 shows a gradual change of water content in meat products in starch-gelatin pouches. The anti-oxidative ability of 200 ppm BHA or 2,000 ppm extract in the film was determined. The starch-gelatin pouch without any antioxidant was served as a control treatment. The properties of

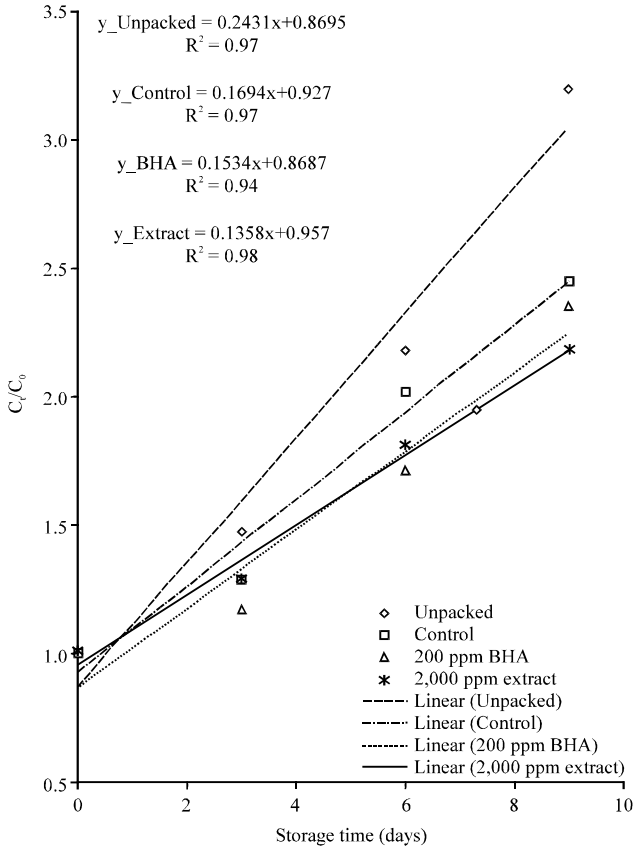

Fig. 3: Evolution of free fatty acid of fried meat ball products during storage

the unpacked meat product were also determined. The trend of water loss of the control product was similar to the unpacked product. The moisture lost rapidly on the 3rd day. At the end of storage period, the extract pouch seems to show moisture protection, while the moisture of the product in BHA pouch decreased continuously. The hydrophilic property of BHA might be considered for the detrimental effect of the moisture barrier of the gelatin-starch film.

Figure 3 shows free fatty acid values in the meat product. Hydrolysis stability of the meat product was generally indicated by free fatty acid development. The FFA, a hydrolysis rancidity parameter, is measured from the hydrolysis reaction of triglyceride. The water molecule basically hydrolyzes the ester groups in the triglyceride molecule to give the products, di-or monoglyceride or glycerol and FFA. Lipase enzyme is sometimes required for a catalyst. Certain FFA molecules (short chain molecule) cause an odor and flavor deterioration of a lipid product. The highest FFA value was observed at the unpacked product. Improvement of hydrolysis stability was found in BHA and extract treatments. The extract treatment showed the slowest rate of FFA development. Antioxidant property of *Kaempferia parviflora* Wall. Ex Baker has been documented, occurring from the flavone compounds (Yenjai *et al*., 2004). Effect of moisture on FFA was not clearly observed, possibly due to a narrow change of moisture content of the products. Therefore, inhibition of lipase enzyme and antimicrobial activity by the antioxidants was responsible for the slowest rate

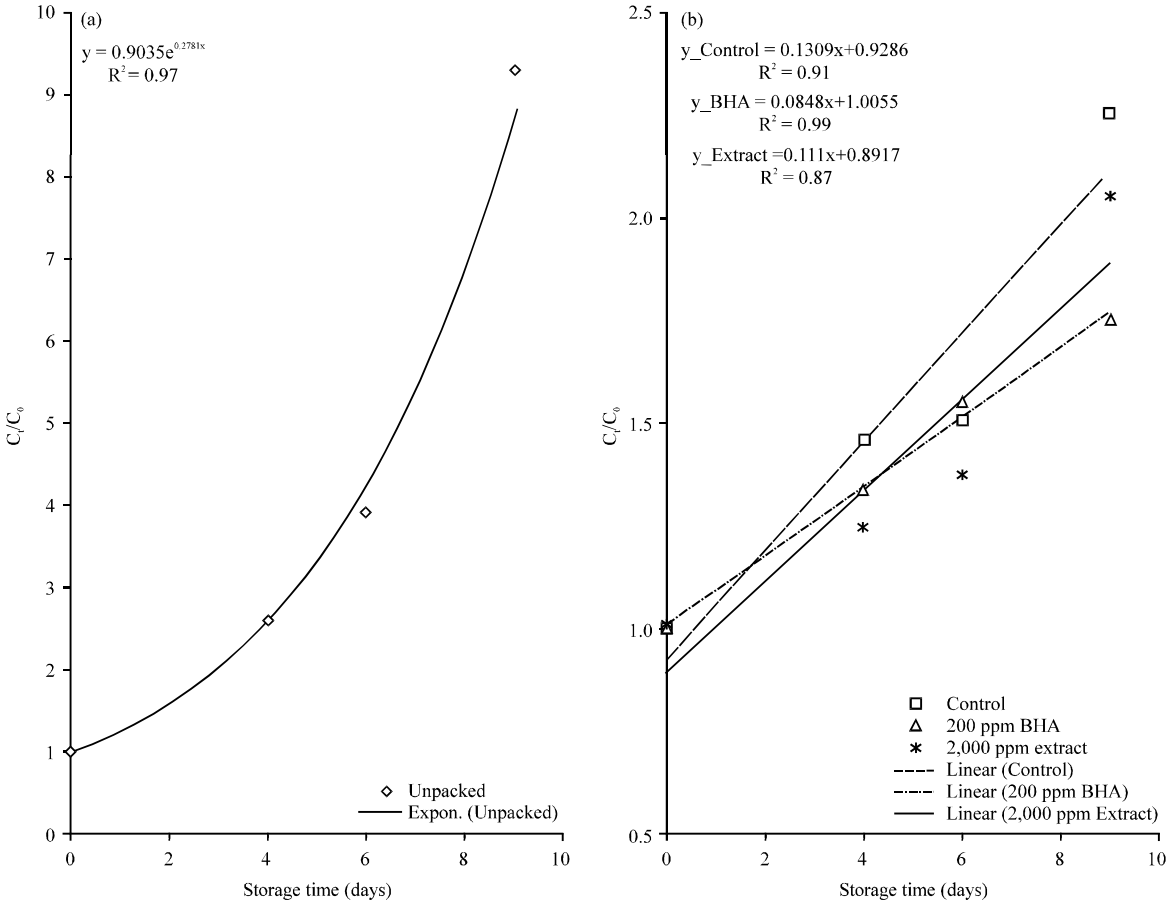

Fig. 4(a-b): Evolution of Peroxide Value (PV) of fried meat ball products during storage, (a) Unpacked and (b) Packed products

of FFA development. Numerous researches have reported that lipid oxidation may be controlled or at least minimized through the use of antioxidants (Sanchez-Escalante *et al.*, 2001, 2003; Gadekar *et al.*, 2014).

Figure 4a-b shows PV values in the meat product. The PV of the unpacked sample was rapidly increased during a 9 day period (Fig. 4a). The PV development of extract treatment was similar to control which was faster than that of BHA treatment. The PV development of the control was unclear at day 6 (Fig. 4b). Peroxide value, an oxidative rancidity parameter, is measured from hydroperoxide content. The hydroperoxide specie generally occurs in the initial phase of oxidative rancidity and then reacts with additional lipid molecules to form other reactive chemical species. The TBA-value should be taken into account to describe oxidation rancidity rate of the products (Fig. 5). However, oxidative stability of meat product, described by using TBA-value was not significantly different within the packed products. Consequently, oxidative rancidity was retarded by gelatin film because of its oxygen barrier property. No conclusion could be reached on the effects of antioxidants given the short storage time.

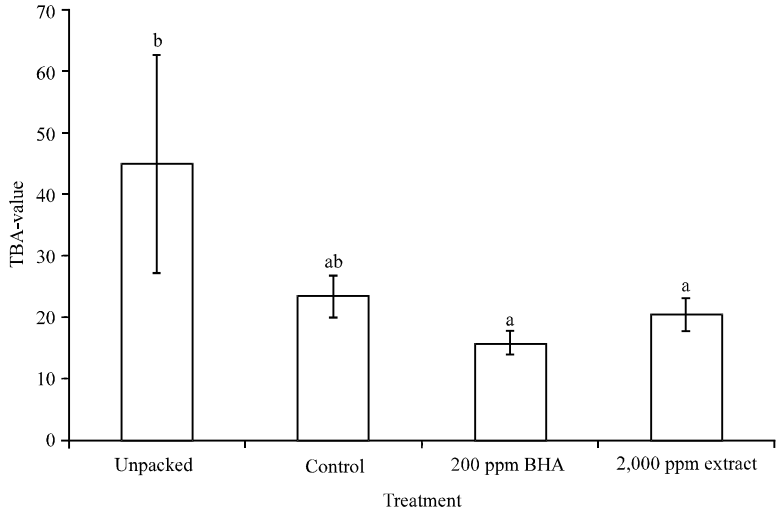

Fig. 5: TBA-value of fried meat ball products during storage

CONCLUSION

So, in conclusion, an appropriate starch portion could significantly improve mechanical property of gelatin films but might contribute to a lower water vapor resistance. Plant extract might modify some mechanical, functional and color properties of gelatin films but it could be a more promising additive to preserve meat products in hydrolytic rancidity (FFA). However, storage time should be extended in order to achieve a clear conclusion in antioxidative ability of the extract.

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