Journal of Food Safety and Food Quality 64, Heft 1 (2013), Seiten 1–32

Arch Lebensmittelhyg 64,
8–14 (2013)
DOI 10.2376/0003-925X-64-8

ISSN 0003-925X

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Incorporation of lactic acid as an antimicrobial agent in polyamide food-packaging films

Einlagerung von Milchsäure als antimikrobiellem Wirkstoff in Polyamidfilmen für die Lebensmittelverpackung

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Summary

With a view to develop an effective antimicrobial packaging method for fresh meat, the utility of a commercial bi-axially oriented polyamide film (15 µm thickness) as a carrier for lactic acid was studied. In a first trial, films were immersed in food-grade lactic acid of various concentrations (10, 20, 40, 80 %) for 70 min, and subsequently dried for 24 h at 40 ± 1 °C and 10% RH. The higher the acid concentration, the higher was the weight gain of the finished films (2.8–10.2 %), whereas films which had been immersed in bidistilled water and then dried, lost 2.5 % weight. Acid uptake was confirmed by thermo-gravimetric analysis. To assess the acid release, lactic acid augmented films were immersed in water for 1 h and 24 h. Equilibrium was reached within 1 h. Acid release was 40–50, ca. 60, ca. 80, and 61–67 µg/cm^2 for 10, 20, 40 and 80 % acid treatment, respectively. Although acid release was highest in 40 % acid treated films, the 80 % acid treated films had the lowest variation in results and were considered for optimizing dipping and drying treatment. Acid release was highest in films which had received acid treatment for 120 min and had been dried for 24 h. Measuring the thickness of cross sections of acid treated films indicated that the distribution of the acid in the film was homogeneous. Relying on the hygroscopic nature of polyamides to allow uptake of organic acids in the packaging matrix is a promising approach to develop novel antimicrobial packaging options for meat.

Keywords: active packaging, lactic acid, polyamide

Zusammenfassung

Im Hinblick auf die Entwicklung eines antimikrobiellen Verpackungsfilms für frisches Fleisch wurde die Verwendbarkeit eines handelsüblichen biaxial gereckten Polyamidfilms (15 µm Dicke) als Träger für Milchsäure untersucht. In einer ersten Versuchsreihe wurden Filme 70 min. in 10, 20, 40, 80%ige Milchsäure (Lebensmittelqualität) getaucht und dann 24 h bei 40 ± 1 °C and 10 % RH getrocknet. Mit steigender Säurekonzentration war auch die Massezunahme höher (von 2,8 auf 10,2 %). In aqua bidest. getauchte Filme verloren dagegen 2,5 % Gewicht. Die Säureaufnahme ließ sich auch durch thermogravimetrische Analyse nachvollziehen. Um die Säurefreisetzung zu bestimmen, wurden milchsäurebehandelte Filme 1 h und 24 h in Wasser immergiert. Der Gleichgewichtszustand wurde nach 1 h erreicht. Die Milchsäurefreisetzung betrug 40–50, ca. 60, ca. 80, und 61–67 µg/cm^2 für in 10, 20, 40 und 80 % Milchsäure getauchte Filme. Die Milchsäurefreisetzung war zwar aus den in 40%iger Säure getauchten Filmen am höchsten, andererseits wiesen die in 80%iger Säure getauchten Filme die geringsten Schwankungen auf und wurden deshalb für weitere Studien zur Optimierung der Tauch- und Trocknungszeiten ausgewählt. Dabei ergab eine Kombination aus 120 min Tauchzeit und 24 h Trocknungszeit die besten Ergebnisse. Durch Dickenmessung von Filmquerschnitten konnte gezeigt werden, dass die Säure gleichmäßig im Film eingelagert wurde. Die Einlagerung organischer Säuren in hygroskopische Kunststoffe, in diesem Fall Polyamid, kann die Grundlage für neuartige antimikrobielle Verpackungen für frisches Fleisch darstellen.

Schlüsselwörter: aktive Verpackung, Milchsäure, Polyamid

Introduction

Rationale for antimicrobial packaging of foods, in particular for fresh meat

The protection of perishable foods such as meat from pathogenic bacteria has become a major issue in times of growing consumer concern about food safety (De Waal, 2003) and high losses of the industry due to recalls (Teratanavat and Hooker, 2004). Even with Good Hygiene Practice (GHP) in place and Hazard Analysis and Critical Control Points (HACCP) systems established, contamination of meats might occur at various processing stages (Paulsen and Smulders, 2003). Since microbial contamination, attachment and growth in meats are primarily surface phenomena, several attempts have been made to apply antibacterial treatments on meat surfaces (Sofos and Smith, 1998; Paulsen and Smulders, 2003). A large variety of physical and chemical treatments of meat has been studied, ranging from irradiation, microwave heating, high-hydrostatic pressure to applications which are more easily integrated in the fresh meat chain, such as hot/cold water washes or sprays (Smith and Graham, 1978; Corantin et al., 2005) or dilute organic acid washes or dips (Smulders and Woolthuis, 1985). Combined steam condensation / water-acid rinse treatments have been reported by Logue et al. (2005) and Smulders et al. (2011, 2012). Depending on the protocol, characteristics of the meat surface, composition and number of the microflora under study, microbial reductions in the range from 1 log to >3 log can be achieved.

In Europe, many of these techniques cannot be applied in fresh meats, as current legislation on fresh meat (EC, 2004) allows only chilling, freezing, shock-freezing, vacuum packaging and controlled atmospheres as preventive measures. However, the latter array of control options fails to eliminate most bacterial pathogens, and occasionally allows their survival (as is the case for *Escherichia coli* O157:H7; Barkoczy-Gallagher et al., 2002) or even their growth (*Listeria monocytogenes;* Walker et al., 1990).

A recent methodology proposed to improve the performance of the predominant forms of meat packaging (vacuum, modified-atmosphere packaging) is the use of 'Antimicrobial Packaging' (AMP) (Vermeiren et al., 1999; Quintavalla and Vicini, 2002), which releases an antimicrobial agent in a controlled and preferably gradual mode to eliminate or reduce contaminant bacteria (Han, 2000).

General considerations on antimicrobial films

In the development of an antimicrobial film, the nature of the polymer, the choice of the antimicrobial, interaction of polymer and antimicrobial compound and release of the antimicrobial into the target matrix have to be considered. For fresh meat in particular, film matrices should provide a good barrier to oxygen, in order to retard spoilage and lipid oxidation (Lawrie and Ledward, 1998), act as a moisture barrier, provide a high puncture resistance for meats with bones, and represent a sealable, inert barrier to the environment and thus preventing recontamination after packaging (Iseppi et al., 2008). A number of polymers has been studied for their suitability as carriers of antibacterial agents, such as cellophane (Hanuskova et al., 2009), polyethylene (Ha et al., 2001), polyamide (Appendini and Hotchkiss, 1997) and polylactic acid (Jin and Zhang, 2008). Antimicrobial agents range from silver nanoparticles (Damm et al., 2008), plant extracts (Ha et al., 2001), lysozyme (Appendini and Hotchkiss, 1997), nisin (Jin and Zhang, 2008) and other bacteriocins (Kim et al., 2002), triclosan (Cutter, 1999) to organic acids (Hanuskova et al., 2009).

Antimicrobials should be homogeneously distributed in or on the related polymer, taking into consideration that not all antimicrobials are sufficiently heat stable to allow blending/co-extrusion with the polymer (Appendini and Hotchkiss, 2002).

The use of organic acids as antimicrobial compounds

Organic acids, their salts and anhydrides have been rather successfully applied in microbial decontamination of carcasses and meat cuts (Van Netten et al., 1994; Smulders and Greer, 1998). Among organic acids, lactic acid (LA) might have the highest consumer acceptance as it is a natural component of meat, approved as food additive and food contact material under European Directive 2002/72/EC (EC, 2002) and because it has a "generally recognized as safe" (GRAS) status (Elias, 1987; Cardenas et al., 2008). Sprays/dips of ≤ 3 % LA are used to sanitize meat surfaces (Shelef, 1994; Smulders and Greer, 1998). The antimicrobial action of organic acids is based on lowering the intracellular pH of bacteria. Organic acids such as LA penetrate the cell membrane in their undissociated form, but will dissociate at higher intracellular pH. To prevent a drop in intracellular pH, excess hydrogen ions have to be exported. This process is energy-demanding and will restrict or even stop cell growth ('metabolic exhaustion'; Cherrington et al., 1991; Shelef, 1994; Bearson et al., 1997).

The efficacy of such organic acids varies between bacterial species. Psychrotrophic meatborne spoilage bacteria – lactobacilli being an exception (Ouattara et al., 1997) – and pathogens *(Listeria, Yersinia)* are generally far more sensitive to organic acids than are mesophilic enteric pathogens (Smulders and Greer, 1998).

Polyamide packaging films

Polyamide films are applied in food packaging, not only because of their good mechanical, thermal, chemical and fatty resistance, but particularly because they serve as a oxygen and aroma barrier (Camacho et al., 2002), and are transparent and brilliant matrices. However, a major drawback of the majority of polyamides is that they are not heat sealable (Kohan, 1995) and have poor moisture barrier properties (Kohan, 1995; Lim et al., 1999) due to their hygroscopic nature (Miri et al., 2009). Yet, the latter is not necessarily disadvantageous. To our knowledge, so far no one has considered to exploit the hygroscopic nature of polyamides for the uptake of polar substances such as lactic acid and to assess their potential as matrix for the incorporation of antimicrobial compounds and to evaluate their future application either as antimicrobial film wrap for fresh meat or as a component of a multilayer film.

Purpose of this study

The purpose of this study was to assess the potential of polyamide films as carrier of lactic acid in terms of acid uptake and release when put in contact with an aqueous environment, with a view to develop an antibacterial meat packaging/wrapping film ("proof-of-concept"), and to optimize lactic acid release from films when immersed in an aqueous environment.

In a following paper, the stability of such lactic acid augmented polyamide films and their antimicrobial effect on vacuum-packed beef will be discussed.

Journal of Food Safety and Food Quality 64, Heft 1 (2013), Seiten 1–32

Materials and Methods

Polymer and antimicrobial compound

Bi-axially oriented polyamide 6 (BOPA6) film (Emblem 15 µm; CFP Flexible Packaging, IT) was chosen as a packaging matrix. Polyamide films were chosen because of their hydrophilic characteristics. The films we used were thin (15 µm), as they were used as inlays in commercial PA/PE films, and should not alter transparency of the whole packaging system.

Food-grade L-lactic acid (PURAC FCC 80; Purac, NL) was used both as a stock solution (80 % w/w according to product specifications) and in diluted form (10 %, 20 %, and 40 %, v/v). Acid concentrations were tested by reversed – phase high – performance liquid chromatography (RP-HPLC) (see below).

Preparation of the antimicrobial film

BOPA6 films were pre-dried as follows: films were loaded into 7 l GENbox jars (bioMerieux, FR) fitted with inert glass devices on which the films were applied. The jar bottom was covered with silica gel. Equilibrium of 3 ± 0.5 % relative humidity (RH) at 24 ± 1 °C was reached after 72 h and registered by a data logger (testo 175-H2, Testo, GE).

Subsequently, films were removed, cut to 9 x 3 cm size and fixed in slots of inert polyethylene devices. Devices were inserted in 1 l glass beakers, were covered with 400 ml of the appropriate LA solution and remained immersed for 70 min at ambient temperature of 22–23 °C, whereafter films were rinsed for 3 s in bidistilled water (100 ml) and stored on inert glass beakers, on which they were dried for 24 h at 40 ± 1 °C (incubator ICE 600, Memmert, GE) and approximately 10 % RH. Drying was applied to ensure that all films used in the experiments were identical in moisture content. According to acid concentrations, films were termed BOPA-L10, BOPA-L20, BOPA-L40 and BOPA-L80.

Mass changes during acid treatment of the film

The weight gain of BOPA6 films assessed after acid immersion treatment and drying by weighing films before and after treatment (analytical balance Sartorius A200S, Sartorius, GE) was compared to the weight changes of a control film, which had been immersed into bidistilled water and dried under the same conditions as described above. It was assumed that the differences between acid-treated and water-immersed films were – as films had been dried after treatment – indicative for the amount of LA incorporated in the film.

Thermo-gravimetric analysis of untreated and acid-immersed films

TGA analysis of BOPA6 and acid-immersed and dried films was carried out employing a TGA Q500 (T.A. Instruments, Waters, US) analyzer and heating up film samples of about 12 mg from room temperature to 200 °C at a heating rate of 5 °C/min in nitrogen atmospheres under a gas flow stream of 50 ml/min.

LA release

LA diffusion from films into bidistilled water was measured (simulating a meat surface environment). To this end, films (9 cm x 3 cm) were immersed in glass tubes containing 10 ml HPLC-grade water (1 film per tube) and subsequently stored in a waterbath (CC1, Huber, GE) maintained at 12 °C. This temperature was chosen as a reference value for future meat trials to evaluate release under boning room – and/or subsequent temperature abuse conditions in the meat chain. After 1 h and 24 h, samples of 100 µl were taken and LA concentration was determined by RP-HPLC.

Determination of LA concentration in bidistilled water

LA was determined by RP-HPLC (Grosheny et al., 1995). The chromatographic system consisted of a Waters Inline degasser AF, a Waters 600 S Controller with an inert Rheodyne 9125 PEEK Injector, a Waters 626 Pump, and a Waters 996 Photo Diode Array detector (Waters). Separation was carried out on a RP-C_{18} column (125 x 3 mm i. d.; 3 µm particle size; ACE, Aberdeen, Scotland), thermostated at 40 °C (Jetstream 2 Column thermostat, VDS optilab, GE). Mobile phase consisted of 2 eluents. Eluent A was composed of 10 mmol/l potassium dihydrogenphosphate (Merck, GE) and 5.5 mmol/l tetrabutylammonium hydrogen sulphate as ion-pairing agent (Sigma-Aldrich, US) in HPLC-grade water. pH was adjusted to 2.5 with orthophosphoric acid (Riedel-de-Haen, GE). Acetonitrile HPLC–S gradient-grade (Fisher Scientific, UK) was used as Eluent B. A step-gradient programme was run with 100% Eluent "A" for 5 min, followed by 95 % "A" and 5 % "B" for 9 min, and finally 100 % "A" for 9 min. Flow rate was 0.7 ml/min. Injection volume was 50 µl. Limit of detection was 0.5 µg LA per injection or 10 µg/ml.

LA standard (0.1 mg/ml) was prepared from an analytical grade L (+) – lactic acid (Fluka, CH) and 3 different volumes (10 µl, 30 µl, 50 µl) were injected in triplicate in order to calculate a calibration curve. LA concentrations were calculated based on peak area.

Modifications of the acid treatment procedure

We studied if prolonged immersion times (2 h, 4 h, 18 h) in LA stock solution and shortened drying periods (5 h, 18 h) at 40 °C and 10 % RH would improve the LA release of BOPA-L80. LA release into bidistilled water was measured by an enzymatic test kit (L-Lactic Acid; r-biopharm, GE) according to instructions of the manufacturer at a wavelength of 340 nm on a spectrophotometer (Hitachi U1100, Hitachi, Japan). To validate the HPLC versus the enzymatic method, 9 samples were tested in parallel with both methods. Concentrations ranged from 110 µg/ml to 220 µg/ml, and the average of differences was 9.4 % (not significant). In the following, films immersed for 2 h in 80 % LA and dried for 24 h will be addressed as BOPA-L80F.

Film thickness

Thickness of cross-sections of BOPA-L80F films was measured by differential interference contrast microscopy (Leica DM 4000 M microscope, objective N PLAN 20x/0.4 BD, equipped with a Leica DFC 320 microscope camera and Leica IM 500, Ver. 4.0.132 software to digitize images; Leitz, GE). Images were in 2088 x 1055 pixel resolution and colour depth was 8 bit per channel. Vertically and horizontally, a 516 pixel distance in the image corresponded to 140 µm. Measurements of 11 x 12 cm films were taken at three cross sections (1 cm from bottom and top, in the middle) per film and performed in triplicate. Per cross section, thickness was assessed at 3 to 4 random locations.

Statistical analysis

ANOVA (SPSS 17.0; SPSS, US), with Fisher s LSD to discriminate among means, was used to assess the influence of the independent factors: 1. "acid concentration", 2. - "duration of immersion in acid during film preparation" and 3. "time for drying of the acid-treated film" on the dependent variables: 1. "mass uptake" and 2. "LA release of BOPA films after 1 h and 24 h immersion in water". LA concentrations obtained by RP-HPLC and enzyme analysis were compared by t-test. Level of significance was set to 0.05.

Results

Film weight and LA uptake

Film weight gains (used as an indicator for LA uptake) and concentrations of LA released by BOPA6 films after 1 h and 24 h of immersion in bidistilled water are presented in Table 1. Weight gain of BOPA6 films was highest when immersed in 80 % LA (BOPA-L80), namely 10.2 % (236 µg/cm² film), followed by films immersed in 40 %, 20 % and 10 % LA, with weight gains of 5.6 %, 3 % and 2.8 %, respectively. All films varied significantly in weight, except for BOPA-L10 and BOPA-L20. Control films that had been immersed in bidistilled water and then dried, lost 2.5 ± 1.9 % weight.

TABLE 1: *Increase in mass during polyamide film preparation and lactic acid release in bidistilled water.*

	Lactic acid concentration %	Increase in mass, n = 10 in %	Increase in mass, n = 10 in µg/cm²	LA release (µg/cm²), n = 6 after 1 h	LA release (µg/cm²), n = 6 After 24 h
BOPA-L10	10	2.8 ± 2.4^{d}	64.9^{d}	38.9 ± 4.8^{a}	50.0 ± 11.5^{ad}
BOPA-L20	20	3.0 ± 1.5^{d}	69.6^{d}	60.7 ± 25.9^{b}	$58.2 \pm v12.6^{acd}$
BOPA-L40	40	5.6 ± 1.3^{c}	129.8^{c}	79.3 ± 11.1^{b}	81.5 ± 8.9^{b}
BOPA-L80	80	10.2 ± 2.1^{b}	236.5^{b}	60.7 ± 10.0^{b}	66.7 ± 0.4^{cd}
	0 (control)	-2.50 ± 1.9^{a}		nd	nd

BOPA6 polyamide films (9 cm x 3 cm x 15 µm) immersed in lactic acid (LA) of various concentrations for 70 min and subsequently dried for 24 h at 40 ± 1 °C and 10 % RH. Within columns, means with superscripts not containing a common letter differ significantly (P ≤ 0.05). nd: not detected.

Extent of LA release

LA release after 1 h immersion into bidistilled water was significantly lower in BOPA-L10 films compared to BOPA-L80, BOPA-L40, BOPA-L20 films. After 24 h immersion, however, release from BOPA-L40 was significantly higher than from other films (Tab. 1). From Table 1, it also can be concluded, that most of the acid was released within the first hour of immersion.

At higher acid concentrations, the differences between weight gain and released LA (Tab. 1) could indicate that water was bound to excess acid within the film matrix and could not be removed by drying at 40 °C. The average amount of released acid was 67 µg/cm² and 81 µg/cm² for BOPA-L80 and BOPA-L40 films, respectively.

Modifications of immersion time and drying time during film preparation

The effect of prolonged immersion times (2 h, 4 h, 18 h as compared to the 70 min) in LA stock solution and shortened drying periods (5 h, 18 h as compared to 24 h) at 40 °C/10 % RH) was assessed to estimate improvements in the LA release. Although BOPA-L40 demonstrated the highest LA release (Tab. 2, 3), BOPA-L80 films were preferred for further tests because they had the lowest variation in results.

A reduction in film weight was observed with prolonged immersion time in LA stock solution (Tab. 4). The effect was more pronounced in films dried only for 5 h, which were found to be sticky at the surface and unsuitable for further meat applications. Films dried for 18 h and 24 h were found to be more promising in regard to film surface characteristics.

LA release from films immersed 1 h and 24 h in water was affected by the duration of acid treatment and drying period. In general, the shorter the duration of acid treatment, the higher the impact of the drying period on the LA release, and the longer the immersion time in LA, the lower the impact of various drying periods (Tab. 2, 3). LA release was highest in films which had received acid treatment for 120 min and had been dried for 24 h. Films obtained according to this "optimised" protocol were termed "BOPA-L80F".

Film thickness

As compared with the untreated polyamide film (15 µm according to product specifications), average film thikness for BOPA-L80F ranged from 17.5 ± 0.7 µm to 18.1 ± 0.5 µm, which indicates a rather homogenous swelling of the antimicrobial film matrix in the order of 2–3 µm. This homogeneity should ensure similar antimicrobial effects across the film.

TABLE 2: *Release of lactic acid into bidistilled water (1 h) from lactic acid augmented bi-axially oriented polyamide 6 films as affected by duration of immersion in lactic acid stock solution and duration of the subsequent drying period (40 °C / 10 % RH), mg/ml, n = 6.*

LA immersion time (min)	Drying time (h) 5	18	24
70	0,10 ± 0,03	$0,12 \pm 0,02^{a}$	$0,16 \pm 0,27^{c}$
120	$0,10 \pm 0,03^{A}$	$0,13 \pm 0,02^{a,A,B}$	$0,17 \pm 0,03^{c,B}$
240	$0,12 \pm 0,03^{C}$	$0,10 \pm 0,02^{b,C}$	$0,11 \pm 0,01^{d,C}$
1080	$0,10 \pm 0,05^{D}$	$0,09 \pm 0,01^{b,D}$	$0,13 \pm 0,01^{d}$

Within columns, means with superscripts not containing a common lowercase letter differ significantly (P ≤ 0.05), in rows, means with superscripts not containing a common capital letter differ significantly (P ≤ 0.05).

TABLE 3: *Release of lactic acid into bidistilled water (24 h) from lactic acid augmented bi-axially oriented polyamide 6 films as affected by duration of immersion in lactic acid stock solution and duration of the subsequent drying period (40 °C / 10 % RH), mg/ml; n = 6.*

LA immersion time (min)	Drying time (h) 5	18	24
70	$0,13 \pm 0,03^{a}$	$0,15 \pm 0,01^{c}$	$0,18 \pm 0,01^{e}$
120	$0,13 \pm 0,04^{a}$	$0,18 \pm 0,01^{c,B}$	$0,19 \pm 0,02^{e,B}$
240	$0,15 \pm 0,02^{b}$	$0,14 \pm 0,02^{d}$	$0,12 \pm 0,01^{f}$
1080	$0,17 \pm 0,07^{b,A}$	$0,14 \pm 0,01^{d,A}$	$0,14 \pm 0,02^{f,A}$

Within columns, means with superscripts not containing a common lowercase letter differ significantly (P ≤ 0.05), in rows, means with superscripts not containing a common capital letter differ significantly (P ≤ 0.05).

Journal of Food Safety and Food Quality 64, Heft 1 (2013), Seiten 1–32

TABLE 4: *Increase in mass (%) of bi-axially oriented polyamide 6 films as affected by duration of immersion in lactic acid stock solution and duration of the subsequent drying period (40 °C / 10 % RH), n = 10.*

LA immersion time (min)	Drying time (h) 5	18	24
70	13,8 ± 2,1^{a}	9,9 ± 3,2^{A}	10,2 ± 2,1^{d,A}
120	12,0 ± 3,1^{a}	8,1 ± 3,1^{c,B}	8,9 ± 0,8^{d,B}
240	9,0 ± 2,6^{b,C}	7,2 ± 1,6^{c,C}	8,3 ± 2,6^{d,C}
1080	6,3 ± 3,5^{b,D}	6,0 ± 2,2^{c,D}	6,4 ± 2,8^{D}

Within columns, means with superscripts not containing a common lowercase letter differ significantly ($P \leq 0.05$), in rows, means with superscripts not containing a common capital letter differ significantly ($P \leq 0.05$).

Discussion

The use of polyamides as carriers of antibacterial compounds

Polyamide has already been tested for its applicability as a matrix to incorporate antimicrobials, such as silver nanocomposites (Damm et al., 2008) or bacteriocins (Kim et al., 2002). In essence, silver and bacteriocins reduced total aerobic counts over a 14 day period by 2 log cycles. Less pronounced effects were reported for polyamide pellets containing lysozyme (Appendini and Hotchkiss, 1997).

A pronounced antimicrobial potential (up to 6 log reduction) of polyamide films as such, after exposure to UV irradiation has been reported (Paik et al., 1998; Shearer et al., 2000), with differences between bacterial species: *Escherichia coli* and *Staphylococcus aureus* were more susceptible than *Pseudomonas fluorescens* and *Enterococcus faecalis* (Shearer et al., 2000).

To the knowledge of the authors, the incorporation of lactic acid in polyamide films as part of active food packaging systems has not been studied.

Weight gain of immersed films

Polyamide films immersed in dilute lactic acids and subsequently dried, demonstrated weight gains ranging from 2.8 to 10.23 %. In principle, four mechanisms could contribute to weight changes during acid-immersion and subsequent drying of polyamide films: 1. weight losses due to extraction of low molar mass chain fragments parallel with/in combination with 2. weight gain due to hydrolysis (Chaupart et al., 1998), and 3. uptake of water, which facilitates 4. the subsequent uptake of acid molecules [this has been proven for an inorganic acid (Kohan, 1995), but it is reasonable to assume that this will apply to organic acids as well]. Considering the chemical stability of polyamides (Abastari et al., 2006), the first and second mechanisms should be of minor importance. Some of the water entering the film will be removed during the subsequent drying process, and finally acid molecules and bound water will remain in the dried film and, thus, contribute to weight gain. This is also affected by concentration and degree of dissociation of the acid solution, which will influence both the water and acid uptake of the polymer. Studies with PA6 (Kohan, 1995) concluded that the rate of water uptake, and thus swelling, is lower the higher the acid concentration. This, in turn, would mean that the weight gain of films immersed in 80 % LA should be mostly due to incorporation of acid molecules; however, from Table 1, it can be concluded that films immersed in 40 % LA released more LA than those with the highest weight gain (i.e. those that had been immersed in 80 % LA). Further studies will be conducted on that issue.

The weight loss of films immersed into bidistilled water has to be further investigated. Although polyamides have a high chemical resistance (Kohan, 1995; Ajji et al., 1998), monomer extraction resulting from immersion in acids cannot be fully excluded and this would deserve further study (EC, 1982, 1985; Silva et al., 2006).

The main issue, i. e. that LA can be incorporated in polyamide films together with water, was also corroborated by preliminary trials with BOPA6 films immersed into 80 % LA and analyzed by thermo-gravimetric analysis (TGA) to determine its fraction of volatile components by monitoring the weight changes that occur as a specimen is heated (Kohan, 1995; Al-Ghamdi et al., 2006). Heating up to 55 °C of untreated BOPA6 (control) film samples lead to an approximate 2 % weight loss, suggesting that a similar effect could be associated with film drying, probably as a result of evaporation of water loosely bound in the amorphous region or present on the surface of the film. In comparison, heating BOPA-L80 films up to 158 °C led to a weight loss of about 9 %. In particular, the weight loss in the temperature range from 55 °C to 158 °C of about 6 %, was – in view of the fact that the boiling point of lactic acid is 119 °C (D Ans and Lax, 1964) – most likely attributable to LA deposited in the film. The total weight loss during heating (9 %) is comparable to the 10.2 % weight gain observed as a result of film immersion in LA (Tab. 1).

Acid release of lactic acid augmented films when immersed into bidistilled water

The films released lactic acid in quantities of approx. 60–80 µg/cm^2, depending on the acid concentration. This is ca $^1/_2$ of the release reported for cellophane films treated with 50 % lactic acid (Hanuskova et al., 2009), which may be caused not only by different chemical composition of the films, but also differences in film thickness. By the same token, the use of polyamide films thicker than 15 µm could also increase lactic acid uptake and release.

Experiments to optimize duration of acid immersion (80 % lactic acid) and drying showed that acid release was highest from films which had been immersed for 120 min and then dried for 24 h, when lactic acid relase both at after 1 h and 24 h is considered (Tab. 2, 3). These films (9 cm x 3 cm) released 1.8 to 1.9 mg in 10 ml bidistilled water (Tab. 2, 3), corresponding to ca. 70 µg/cm^2. However, when only the release after 24 h is considered, films having received acid immersion times of 70 min and 120 min, and a drying time of 24 h did not differ significantly; liekewise, films having received a 120 min acid immersion, and drying periods of 18 h and 24 h did not differ significantly in acid release capacity (Tab. 3).

When such films are used for meat packaging, the concentration which can be achieved on the meat surface will depend on the thickness of the exudate film covering the meat surface (see below).

Film thickness

As compared with the untreated polyamide film (15 µm according to product specifications), average film thickness for BOPA-L80F ranged from 17.5 ± 0.7 µm to 18.1 ± 0.5 µm, which indicates a rather homogenous swelling of the antimicrobial film matrix in the order of 2–3 µm. This homogeneity should ensure similar antimicrobial effects across the

film. The latter is considered of high importance as the thikness of the meat exudates film covering the meat surface can vary considerably (Den Hertog-Meischke et al., 1997). Thus, one must ensure that film thickness is standardized to afford a similar release to muscle surface exudates and to avoid further variability of antimicrobial effects of the film. BOPA-L80F films were found promising in this respect.

Conclusion

Lactic acid can be incorporated in food-grade polyamide films by a simple immersion procedure. Films of 15 µm thickness were able to release lactic acid in amounts of approx. 60–80 µg/cm² film. Such films could be used as the inner layer of a multilayer vacuum-packing film to be used in fresh meat industry. Considering the chemical stability of polyamide films, the incorporation of lactic acid and water should have little effect on mechanical stability, and in a multilayer package the mechanical and barrier functions would be represented by other layers anyway. Further studies will be conducted on that issue as well as on the question how significant the effect of lactic acid released by the films into a meat (-juice) environment will be in terms of reduction of contaminant bacteria.

Acknowledgments

This study was conducted in the framework of the EU-Project FOOD-CT-2006-36241 (ProSafeBeef). The support of Dr. Madai and Dr. Mumelter from mondi flexible packaging is gratefully acknowledged. Additionally, thanks go to Dr. Tichy for the help in statistics.

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