Impact of Biodegradable PHB Packaging Composite Materials on Dairy Product Quality Biodegradējamo PHB iepakojuma kompozītmateriālu ietekme uz piena produktu kvalitāti

Sandra Muizniece-Brasava, Lija Dukalska

Faculty of Food Technology, LLU, e-mail: sandramlv@yahoo.com LLU Pārtikas Tehnoloģijas fakultāte, e-pasts: sandramlv@yahoo.com

Abstract. Poly-\(\beta\)-hydroxybutyrate (PHB) composite materials (plasticizers: dioctylsebacate, bisoflex) synthesized at the University of Latvia Institute of Microbiology and Biotechnology in laboratory conditions and commercially produced in Brazil, moulded out at the Riga Technical University Institute of Polymer Materials, were used for sour cream packaging in pouches with dimensions 90x90 mm. The impact of those materials on quality indices of sour cream was compared to materials traditionally used for dairy product packaging: Lean Pouch, PE covered with light protective graphite layer films, and PS cups of 250 ml volume. The packages were stored for 18 days under fluorescent lighting at the temperature of $+5\pm0.5$ °C. Quality indices: colour, pH, and secondary lipid oxidation products were determined. Calcium and iron migration from new and for sour cream packaging used PHB composite materials to simulant at pH 4 and pH 7 was studied. Tristimulus Colorimeter measured colour changes: colour Tec PCM/PSM (CIELab system). Secondary lipid oxidation product dynamics during storage were determined by headspace GC according to the method of Shahidi and Pegg with minor modifications. Oxidation product contents in samples were quantified calculating the peak area of the product relative to those of the internal standard. Fe and Ca migration from PHB composite materials and Lean Pouch film to simulant was determined by atomic absorption spectrometer "Shimadzu AA-6701F". Comparison of migration results at different pH values showed that ambient acidity considerably influences ion migration. Kind of plasticizer influences Ca and Fe migration from new and used packaging materials as well. PHB composite materials were found as suitable packaging materials for sour cream relatively similar to conventional plastics, inconsiderably affecting quality changes during storage.

Key words: PHB, colour, migration, secondary lipid oxidation, quality changes.

Introduction

The interaction between synthetic polymers and natural environment in terms of the effects of oxygen, radiant energy, and living organisms has been extensively studied over the last years. However, recent trends in preservation of the environment have created much public interest. This is due to the heavy usage of plastics as packaging materials in consumer industry (Aminabhavi, Balundgi, 1990). At present, viewpoints on polymer materials vary rapidly. The current legislation, socially economical and social trends in Europe promote the regularly increasing usage of renewable raw materials, used package recycling and composting. The aim of European Environmental Bureau (EEB) is to improve environmental problems in European Union countries, related to packaging waste. Currently, various biodegradable polymers are combined to improve technical performance of the final products, to open up new applications or to cut costs. In general, biobased polymers may be divided into three categories based on their origin and production (Petersen et al., 1999):

- Category 1: polymers directly extracted/removed from raw materials – regularly renewable biomass.
 Examples are polysaccharides such as starch and cellulose, and proteins like casein and gluten;
- Category 2: polymers produced by classical chemical synthesis using renewable biobased monomers. An example could be polymer fermented from organic substances, for example, lactic acid – polylactate (PLA);
- Category 3: polymers produced by microbial synthesis or genetically modified bacteria, for example, polyhydroxyalkanoates (PHA), of which polyhydroxybutyrate (PHB) is the most common.

The specific use of renewable raw materials (RRM) such as corn, potato, wheat and other carbohydrate sources as feedstock for the production of RRM-based polymers can give the final products certain advantages in terms of reduced environmental impact. These include a reduction in the use of fossil resources and reduced CO_2 emissions, energy content and waste disposal problems. However, the real strength of these RRM-based materials is their biodegradability and

compostability when used in packaging applications. The basic idea behind biodegradable polymers is taken from nature's cycle. Worldwide, more than 60 billion tons of organic material is generated every year by photosynthesis. Most of it is subsequently converted back into the starting products, carbon dioxide and water, by microorganisms. This cycle is the role model for biodegradable plastics (BDPs), which are often made from renewable raw materials obtained from agricultural production (The Kassel ..., 2005).

Biodegradable polymers especially those derived from biological sources are a minor factor in the world plastics market. A recent report on bio-based degradable polymers by the European Commission's Institute for Prospective Technological Studies concluded that these materials would have a 1 to 2% market share of all polymers in Europe by 2010 or 0.5 to 1.0 million tons, and no more than a 5% share by 2020 - 3 to 5 million tons. In 2002/2003, the manufacturing capacity of PHB from all biodegradable packaging amounts in the world was about 2%. During the previous three years, biopackage market has remarkably developed in the scale of the whole world. A continuous growth of the bioplastics sector is foreseen in the following 10 years, renewable raw materials being the main feedstock (International ..., 2005).

As the development of biobased and biodegradable packaging materials has progressed rapidly within the last decade, some of those are already competitive alternatives to conventional food packaging materials. Among the alternatives, polyhydroxybutyrate (PHB) and polylactate (PLA) offer high potential for food packaging applications (Sinclar, 1996; VanTuil et al., 2000) due to various properties, matching those of conventional packaging materials (Sinclar, 1996; Hanggi, 1995; Krochta, DeMulder-Johnston, 1996; Petersen et al., 2001; Haugaard et al., 2003). Based on these properties, the use of PLA and PHB has been suggested for several foods including beverages such as orange juice and some fatty foods (Haugaard et al., 2001; Weber et al., 2002). However, in-depth knowledge on the suitability of PLA and PHB for foods is still limited.

Polyhydroxyalkanoates (PHA), of which polyhydroxybutyrate (PHB) is the most common, can be accumulated by a large number of bacteria as energy and carbon reserves. Due to their biodegradability and biocompatibility, these optically active polyesters may find industrial applications. The properties of PHA are dependent on their monomer composition and therefore it is of great interest that recent research has revealed that, in addition to PHB, a variety of PHA can be synthesized with bacteria. The monomer composition of PHA depends on the nature of the carbon source and the microorganism used. PHB is a typical highly crystalline thermoplastic whereas long-side chain PHA is elastomer with low melting points (Eggink et al., 1994). In Latvia, biodegradable poly-β-hydroxybutyrate (PHB) and its derived composite materials were synthesized and recovered from the biomass of *Azotobacter chroococcum 23* (Savenkova et al., Patent LV No. 5297, 1993) with a polymer content above 75% of the dry cell weight (Savenkova et al., 1999). PHB was extracted from isopropanol-pretreated biomass by hot chloroform and precipitated with isopropanol. Its PHB composite material barrier properties have been investigated at the Latvia University of Agriculture (Muizniece-Brasava, Dukalska, 2005).

Another kind of sustainable packaging material – Lean Pouch (manufactured by Ecolean company), the degradable plastic material consisting of PE mixed with at least 40% calcium carbonate (chalk) – is used for dairy products and juice in Sweden (Packaging ..., 2002; Fuldman, 2002). Lean Pouch is photodegradable. This process can technically be speeded up with the help of degrading agents. Tests in China have shown that with additions of degrading agent the material can be classified (Ecolean ..., 2003; More ..., 2003).

In order to increase the knowledge on the impact of environmentally friendly packaging materials on some dairy products, the aim of the present work was to investigate the suitability of biobased PHB composite materials synthesized and produced in two different countries (Latvia and Brazil) for packaging, as well as compare their impact on quality with usually for dairy product packaging used conventional plastic materials: developed from Ecolean company material Lean Pouch (LP) and polyethylene (PE) films covered with graphite light protective layer, as well as PS cups. Sour cream has been chosen for study. Different changes in quality indices occur during the storage of sour cream providing a wider overview of the packaging material impact on foods. In case of packaging of fatty foods, such as sour cream, it is important to avoid colour changes (Manheim et al., 1987; Gvozdenovic et al., 2000) and minimize lipid oxidation and generated off-flavours (Bekbolet, 1990; Hansen, Skibsted, 2000; Lenersten, Lingnert, 2000; Giese, 2000).

Materials and Methods

Materials. Poly- β -hydroxybutyrate (PHB) synthesized at the University of Latvia Institute of Microbiology and Biotechnology under laboratory conditions and commercially produced in Brazil were used for experiments. PHB composite material films were moulded out at the Riga Technical University Institute of Polymer Materials and used for sour cream packaging in pouches with dimensions 90x90 mm. Dioctylsebacate (DOS) and Bisoflex (B) were used as PHB plasticizers. Following packaging materials were used for experiments:

- PHB+DOS (77% + 23%) (PHB synthesized in Latvia) (film thickness $35 \pm 3 \mu m$);
- PHB+DOS (77% + 23%) (PHB commercially produced in Brazil) (film thickness 35 ± 3 μm);

- PHB+B (77% + 23%) (PHB commercially produced in Brazil) (film thickness 35 ± 3 μm);
- PHB+DOS (77%+23%) + increased Fe content (PHB synthesized in Latvia) (film thickness 35 ± 3 μm);
- (PE) polyethylene film covered with light protective graphite layer (film thickens 35 ± 3 μm);
- (LP) Lean Pouch (film thickness $78 \pm 5 \,\mu$ m);
- (PS) polystyrol cups, volume of 250 ml (thickness $270 \pm 10 \,\mu$ m).

The thickness of films was measured by digital micrometer (Electronic Digital outside Micrometer, Q478) to the nearest 0.001 mm at 5 locations.

Food product for study: sour cream with 18% fat content produced at holding company "Straupe", Latvia.

Storage. A total number of 14 samples for each of the 7 different packaging materials were prepared. The packed sour cream samples were stored in Commercial Freezer/Cooler ELCOLD at $+5\pm0.5$ °C temperature (controlled by MINILog, Gresinger electronics) 18 days under fluorescent lighting (OSRAM Lumilux De Luxe) with a radiant fix at 100-800 lux (measured by light meter LX-107). Throughout the storage period, the samples were randomly interchanged to minimize unequal temperature fluctuations and light conditions. At each time of measurement, two identical packages were analyzed. The results were reported as averages of these two determinations. On days 0 (before packaging), 2, 4, 7, 10, 15, and 18, color and pH changes as well as secondary lipid oxidation products were determined.

Methods. In our experiments, the quality changes were characterized by measurement of colour, pH values and formation of secondary lipid oxidation products in the sour cream, as well as by Ca and Fe migration from PHB composites to the simulant.

- Tristimulus Colorimeter measured Hunter colour parameter changes: Colour Tec PCM/PSM (CIEL*a*b* system). Colour values were recorded as L* (lightness, 0 = black, 100 = white). Values of a* (-a* = greenness, +a* = redness) and b* (-b* = blueness, +b* = yellowness) are the two chromatic components which range from -120 to +120 (Papadakis et al., 2000). Glass Petri dishes (Ø 75 ×15 mm) were filled with the sour cream samples. The measurements were repeated on five randomly selected locations on each sample dish.
- pH values of the sour cream samples were determined by 720 pH-meter using an electrode SenTix 81 (3 mol/KCl).
- Secondary lipid oxidation products in sour cream were determined by headspace GC according to the method of Shahidi and Pegg (Shahidi, Pegg, 1994) with minor modifications. Sour cream (2.0 g) was accurately weighted into 20 ml vials and 5methyl-2-hexanon was added as an internal standard to yield 100 ppm w/w. The samples were preheated in HP 7694 Head Space Sampler (Hewlett

Packard, Palo Alto, CA, USA) for 45. min at 90 °C before the vapor phase was transferred to a 3 cm³ loop (321–056 HSP) under the following conditions: carrier gas-helium, vial pressure-0.90 bars, pressurization time on the vials -0.13 min., loop fill time -0.04 min, loop temperature -100 °C, transfer line temperature - 110 °C, loop equilibration time -0.02 min. Chromatographic separation was performed by a PH 6890 GC-Head Space (Hewlett Packard, Palo Alto, CA, USA). From the loop, the vapor phase was injected (injection time -0.40 min, injection temperature - 195 °C) onto a high polarity HP19095X-123HP – polyethylene glycol column was bonded $(30.0 \times 530 \,\mu\text{m} \times 1 \,\mu\text{m})$. Helium was the carrier gas employed at an Intel pressure of 0.65 bars with a split ratio of 7:1. The oven temperature programming comprised three steps: $35 \degree C (1 \text{ min.})$, 55 °C (1 min.) after heating at 5 °C min.-1, followed by 175 °C (1 min.) after heating at 10 °C min.⁻¹. The flame ionization detector (FID) temperature was 250 °C. The contents of secondary lipid oxidation products in samples were quantified by calculating the peak area of the product relative to that of the internal standard.

 Ca and Fe migration from new and for sour cream packaging used material was determined by Atomic Absorption spectrometer "Shimadzu AA-6701F" according to "The Standard Methods 3111: Metals by Flame Atomic Absorption Spectrometry" (Standard Methods ..., 1998) (simulants – 0.07 M HCl, pH 4.0 and distilled water, pH 7.0).

Statistical analyses. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS 10.0 (Arhipova, Bāliņa, 2003). Two-way analyses of variance ($p \le 0.05$) were used to determine significance of differences between means of colour, pH and secondary lipid oxidation. Compare Means, One Way Anova ($p \le 0.05$) were used to determine significance of differences between means of the calcium and iron migration.

Results and Discussion

The colour of dairy products changes during storage under the influence of lighting. This change might be due to the photo destruction of some of the brightly coloured constituents such as riboflavin, β -carotene and vitamin A, directly influencing the colour components a* and b* according to Hunter. It may also be explained by the modification of the light-scattering structure of dairy product (photo agglomeration, photolysis, etc.), which has a direct influence on the colour component L* (L-value in Fig. 1) and an indirect influence on colour components a* and b*. Light exposure of different unpackaged dairy products (skimmed, raw, pasteurized, condensed milk, coffee cream, sour cream) resulted in a decrease in colour com-



Fig. 1. Changes in colour L*-, a*-, and b*-values in sour cream during storage.

ponent L* (less brilliant), an algebraic increase in the negative component a* (less greenness), and decrease in the positive component b* (less yellowness) (Bosset et al., 1994).

The lightness considerably increases in the first 4 storage days. Experimentally it was observed that during the first two storage days a*-value for all samples decreased very noticeably from -0.8 to -(2.3-2.7) (less green, similarly as Bosset has found) (a-value in Fig. 1). A follow-up experiment up to 18 days did not show any significant differences in a*-values (p>0.05). At the same time, a*-value of samples packaged in Lean Pouch film differed from the ones packed in PS cups and PHB+DOS+Fe (Latvia) ($p \le 0.033$). The dynamics of b*-value at the storage time of 18 days, contrary to Bosset results for unpacked dairy products, in our experiments changed from 8.0 to 8.6-9.1 (more yellow) (bvalue in Fig. 1). A significant difference was found for b*-values among the sour cream samples packaged in PHB+DOS composite materials synthesized and produced in Latvia and Brazil (p=0.007). PHB+B composite material with 95% probability substantially variously influenced the b*-value of sour cream samples compared to those packaged in all other types of the investigated materials ($p \le 0.049$). The b*-value of sour cream packaged in Lean Pouch film was disparate from PHB+B (p=0.004). A significant difference in yellowness was

found to the sour cream packaged in commercially used PS cups and PHB+DOS (Latvia) and PHB+B (Brazil) ($p\leq0.05$).

Significant differences in pH values during storage time of 18 days were found among all sour cream samples packed in different kinds of materials (p<0.05) (Fig. 2). The pH value of sour cream samples in PHB+B (Brazil) and PHB+DOS (Latvia and Brazil) composite materials with probability of 95% substantially varied from those packed in Lean Pouch film, PHB+DOS+Fe (Latvia) films, and PS cups (p≤0.036). A significant difference in pH values was found for samples packaged in commercially used PS cups and PHB+DOS+Fe (Latvia) compared to all other investigated samples.

pH value of sour cream packaged in PHB composite materials and Lean Pouch film (p≤0.036) significantly differed from pH value of sour cream packaged in PHB+DOS+Fe pouches (Latvia) and PS cups. On the contrary, pH values of samples packaged in Lean Pouch film were disparate from the results obtained for sour cream packaged in all other kinds of investigated materials (p≤0.026). This phenomenon could be explained by calcium migration from film to sour cream, as a result of which Ca lactate is being formed from lactic acid and pH value increases.

The secondary lipid oxidation products acetaldehyde, pentanal, 2-methyl-1-propanol and 1-butanol



Fig. 2. Dynamics of pH values.

(Fig. 3) were detected in sour cream samples packaged in various materials chosen for the experiments. Significant differences in acetaldehyde, pentanal and 2methyl-1-propanol among sample types and storage time (p<0.05) were observed. One of the secondary lipid oxidation products – 1-butanol – was not found in the investigated samples on 0 day; its formation at the storage time was not ascertained as well.

A significant difference in acetaldehyde quantity was found for sour cream packaged in PHB composite

materials with plasticizer DOS (Latvia and Brazil), compared to Lean Pouch, PE with light protective graphite layer, and PHB+B (Brazil) films ($p\leq0.035$). Acetaldehyde content in sour cream samples packaged in PHB+B (Brazil) with probability of 95% substantially varied from those packaged in PHB+DOS and PHB+DOS+Fe composite material films synthesized in Latvia ($p\leq0.035$). The influence of Lean Pouch and Polyethylene films, covered with graphite light protective layer, on the increase of acetaldehyde amount in sour



Fig. 3. The content of acetaldehyde, pentanal, and 2-methyl-1-propanol in sour cream packaged in different packaging materials and stored under fluorescent lighting.

Table 1

	Acetaldehyde,		Pentanal,		2-methyl-1-propanol,	
Package types	mg kg ⁻¹		mg kg ⁻¹		mg kg ⁻¹	
	0 day	15th day	0 day	15th day	0 day	15th day
PHB+DOS (Latvia)	1.17±0.06	13.34±0.67	0	0.045 ± 0.002	0	0.100±0.005
PHB+DOS (Brazil)	1.17±0.06	11.10±0.56	0	0.030±0.002	0	0.080 ± 0.004
PHB+B (Brazil)	1.17±0.06	6.89±0.35	0	0	0	0.070 ± 0.004
PHB+DOS+Fe (Latvia)	1.17±0.06	16.47±0.82	0	0	0	0.130±0.007
Lean Pouch	1.17±0.06	2.46±0.12	0	0	0	0
PE with light protective graphite layer	1.17±0.06	1.47±0.07	0	0	0	0

The dynamics of acetaldehyde, pentanal, and 2-methyl-1-propanol in sour cream within 15-day storage

cream samples with 95% probability substantially varied compared to the results of samples packaged in all other kinds of investigated PHB+DOS composite materials ($p\leq0.004$). PHB+DOS composite materials synthesized and produced in Latvia and Brazil with probability of 95% substantially variously influenced the pentanal amount in sour cream samples compared to those packaged in all other kinds of investigated materials (p0.012).

The value of 2-methyl-1-propanol in sour cream samples packaged in PHB+DOS (Latvia and Brazil) composite materials with 95% probability substantially varied from those packaged in Lean Pouch and Polyethylene film (PE) covered with light protective graphite layer ($p\leq0.022$). The influence of Lean Pouch, PE covered with light protective graphite layer and PHB+B produced in Brazil on the 2-methyl-1-propanol dynamics in sour cream is disparate from all investigated PHB composite materials synthesized in Latvia (p=0.035). A significant difference in 2-methyl-1-propanol changes was found for sour cream samples packaged in PHB+DOS+Fe (Latvia) from Lean Pouch and commercially used PE covered with light protective graphite layer films ($p \le 0.002$).

Experimentally it was established that at the storage time of 15 days changes in secondary lipid oxidation products for samples packaged in Lean Pouch and commercially used PE covered with light protective graphite layer films, compared to threshold values, did not differ significantly. On the contrary, in the samples packaged in all investigated PHB composite material pouches those values increased (Table 1). It could be explained by a 1.5 times higher light transmission of PHB composite materials compared to Lean Pouch film. Light transmission of polyethylene film (PE) covered with light protective graphite layer is close to zero.

The kind of PHB plasticizer influences calcium migration to simulant at pH 7 during 10 days ($p\leq0.001$) (Fig. 4). Obviously, the amount of calcium migrated from a new and for sour cream packaging used material essentially increased ($p\leq0.001$) and, consequently, pH value of the product influenced material structure. As Lean Pouch material consists of PE mixed with at least 40% calcium carbonate, the amount of migrated cal-



Fig. 4. Calcium migration to simulant at pH 7 within 10 days.

cium is noticeably higher (Fig. 5) than that migrating from PHB composite materials. pH values don't influence the calcium migration from material to simulant (p>0.05), however, the amount of calcium migrated from a new and for sour cream packaging used material is disparate both at pH 7 and pH 4 (p≤0.001). At the polyβ-hydroxybutyrate (PHB) synthesis process, Azotobacter chroococcum 23 accumulate iron ions in the cells and, by recovering PHB from the biomass, iron ions dispose of cells and partially mix with PHB. Whereas the kind of PHB plasticizer does not influence iron migration to simulant at pH 7 (Fig. 6a) (p>0.05). In the same way, reduced ambient sourness (pH 4) (Fig. 6b) influences the migration from all investigated PHB composite materials (p≤0.003). The amount of iron migrated from a new and for sour cream packaging used material was not observed at pH 7 (p>0.05), while at pH 4 the migration amount was disparate ($p \le 0.003$).

Conclusions

1. Significant differences in L^* -, a^* - and b^* -values during 18 days of storage were found among all sour cream samples packaged in different kinds of materials; the kind of PHB plasticizer slightly influences L^* -, a^* - and b^* -values during the storage.



Fig. 5. Calcium migration from Lean Pouch material to stimulant within 10 days.

2. Significant differences in pH values during the 18 day-storage exist among all sour cream samples packaged in different kinds of materials.

3. A significant difference in acetaldehyde, pentanal and 2-methyl-1-propanol content among sample types and storage time was observed.

4. The kind of PHB plasticizer influences calcium migration from material to simulant at pH 7, and the amount of calcium migrated from the new and for



Fig. 6. Iron migration to simulant within 10 days: a - at pH 7; b - at pH 4.

sour cream packaging used material differs significantly.

5. The kind of PHB plasticizer does not influence iron migration to simulant at pH 7; reduced ambient sourness influences the migration from all investigated PHB composite materials; at pH 4 the migration amount is disparate for the new and for sour cream packaging used material.

6. PHB-based polymer films with various plasticizers (Dioctylsebacate or Bisoflex) might be suitable for different packaging technologies of dairy products.

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Anotācija

Latvijas Universitātes Mikrobioloģijas un Biotehnoloģijas institūta laboratorijā sintezēts un Brazīlijā rūpnieciski ražots poli-β-hidroksibutirāts (PHB) un no tā Rīgas Tehniskās universitātes Polimēru Materiālu institūtā veidoti kompozītmateriāli (plastifikatori *dioctylsebacate* un *bisoflex*) ir lietoti skābā krējuma iepakošanai spilvenveida pakās (90x90 mm). Salīdzināta PHB kompozītmateriālu (plēves biezums 35±3 µm) un komercsistēmā piena produktu iepakošanai lietoto materiālu (ar gaismas necaurlaidīgu grafīta kārtu pārklātu PE, biezums 35±3 µm un Lean Pouch, biezums 78±5 µm plēves, kā arī no PS izgatavotas glāzītes, tilpums 250 ml, biezums 270±10 µm) ietekme uz produkta kvalitātes izmaiņām, uzglabājot 18 dienas +5±0.5 °C temperatūrā fluorescējošā apgaismojumā. Analizēti kvalitātes rādītāji: produkta pH, krāsas (CIELab sistēmā) izmaiņas un sekundārās lipīdu oksidācijas produktu dinamika, kā arī kalcija un dzelzs migrācija uz simulantu (pH 4 un pH 7). Eksperimentu analīze rāda, ka PHB kompozītmateriāli ir lietojami piena produktu (skāba krējuma) fasēšanai.