

**INFLUENCE OF PACKAGING MATERIAL AND STORAGE TIME  
ON PHYSICAL, CHEMICAL AND MICROBIOLOGICAL  
PROPERTIES OF SET YOGURT: A COMPARATIVE STUDY  
BETWEEN MODIFIED BIODEGRADABLE POLY(LACTIC ACID)  
AND POLYPROPYLENE**

NAWADON PETCHWATTANA<sup>1\*</sup>, PHISUT NAKNAEN<sup>2</sup>

<sup>1</sup>Division of Polymer Materials Technology,

<sup>2</sup>Division of Food Science and Nutrition,

Faculty of Agricultural Product Innovation and Technology,  
Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110 Thailand

\*Corresponding Author: nawadon@g.swu.ac.th

**Abstract**

The current paper investigates the influence of storage time and type of polymeric packaging material on the chemical, physical and microbiological properties of set yogurt. Firstly, poly(lactic acid) (PLA) was modified by using a core-shell rubber (CSR) and an acrylic processing aid (PA) to produce PLA with high toughness and good processability. Secondly, an appropriate PLA/PA/CSR composition was selected and fabricated to yogurt cup. The yogurt was stored in both modified PLA and polypropylene (PP) packages to observe some physical, chemical and biological changes. Finally, the biodegradation test was made on both packages and compared with that cellulose. Experimental results revealed that adding 5wt% CSR gave PLA/PA as tough as PP. Types of packaging material and storage time did not change the color of yogurt. The number of lactic acid bacteria grew significantly after they had been incubated for 6 days. The bacterial viability decreased dramatically due to the increased acidity and the decreased pH. A positive impact on the viability of bacterial growth was found when yogurt was stored in modified PLA package. This made yogurt had more health benefits than stored in PP package. The biodegradation test results indicated that the modified PLA degraded at a rapid rate. It achieved approximately 50% biodegradation within 40 days which was comparable to the time required to degrade the cellulose, whereas PP was non-biodegradable over the period studied. In summary, substitution conventional PP by a novel modified PLA seems to be a better way for both the health and the environment benefits.

Keywords: Biodegradable polymer; Food quality; Set yogurt; Biodegradation.

## Nomenclatures

$a^*$	Redness and Greenness
$b^*$	Yellowness and Blueness
$L^*$	Lightness
$T_g$	Glass transition temperature, °C

## Abbreviations

ANOVA	Analysis of Variance
AR	Acrylate rubber
ASTM	American Society for Testing and Materials
CRD	Completely Randomized Design
CSR	Core-shell rubber
EGMA	Poly(ethylene-glycidyl methacrylate)
ISO	International Organization for Standardization
LDPE	Low Density Polyethylene
MFI	Melt Flow Index, g/10min
MRS	de Man, Rogosa and Sharpe
OTR	Oxygen Transmission Rate, ml/m <sup>2</sup> .24days
PA	Processing aid
PCL	poly( $\epsilon$ -caprolactone)
PET	Poly(ethylene terephthalate)
PLA	Poly(lactic acid)
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PS	Polystyrene
RH	Relative Humidity, %

## 1. Introduction

Nowadays, fossil based plastics have been extensively utilized as food packaging materials due to their availability, processability, good mechanical and thermal stabilities and low cost [1-2]. However, the disposal of petroleum based plastics products also contribute significantly to environmental problems due to their non-biodegradability [3-4].

For disposal issue, conventional petroleum based plastics are being substituted with biodegradable and renewable materials [5-6]. Poly(lactic acid) (PLA) is an example of the biodegradable materials which have drawn more attention from both industries and research institutions [7-8]. Although PLA has been known for more than a century but it has only been of commercial interest in recent years. PLA offers numerous advantages such as biodegradability, recyclability, renewability, commercial availability and good processability [8-10]. However, brittleness, low resistance to impact and extremely low crystallization rate are the major parameters restricting the use of PLA in many value-added applications [1, 8-10]. These have motivated many researchers to toughen PLA prior to utilize as food packaging. Petchwattana *et al.* [4] toughened PLA with ultrafine fully vulcanized acrylate rubber (AR). Adding 10wt% AR made PLA tougher with the

increased tensile elongation at break and impact strength by 40 and 4 times respectively. Cabedo *et al.* [11] found an improvement in toughness and gas barrier properties of the film prepared from PLA/poly( $\epsilon$ -caprolactone) (PCL)/nanoclays hybrid composites. During storage, Koide and Shi [12] found insignificant differences in the color, weight loss, hardness and ascorbic acid content of the green peppers stored in PLA and low density polyethylene (LDPE). However, PLA containers were found to be the cause of a faster wine quality loss compared to poly(ethylene terephthalate) (PET) and glass due to its higher moisture permeability [13].

This study aims to examine the possibility of using modified PLA as a biodegradable package for set yogurt. The impact modified PLA and polypropylene (PP) packages were fabricated through sheeting and thermoforming process used for making yogurt cup. The performances of each package were evaluated by means of biological, physical and chemical properties. A set yogurt was selected as a food simulant for the PP and the modified PLA cups. To extend the study, further evaluation was made by observing the biodegradation of both packages and compared to cellulose.

## 2. Experimental Works

### 2.1. Materials

An extrusion/thermoforming grade of PLA (PLA2003D, NatureWorks LLC) was used as a polymer matrix. Its melting range and density at room temperature were 160-170°C and 1.24g/cm<sup>3</sup> respectively. Fine particles of core shell rubber (CSR) (Paraloid<sup>TM</sup> BPM-515, Dow Chemical, USA) were applied to the PLA for toughening purpose. The glass transition temperature ( $T_g$ ) of the acrylic rubber core and the poly(methyl methacrylate) (PMMA) shell were -40 and 105°C respectively. The acrylic processing aid (PA) (Paraloid<sup>TM</sup> BPMS-260, Dow Chemicals, USA) was applied to PLA for rheological modification purpose. Its density and average particle size were 0.45g/cm<sup>3</sup> and 200nm respectively. A thermoformimg grade PP (Moplen HP748H, HMC Polymers PLC) was selected as a conventional yogurt cup reference. Table 1 shows the blend formulations of PLA, PA and CSR.

**Table 1. Formulations of PLA, PA and CSR.**

<b>Formulation code</b>	<b>Material compositions (wt%)</b>			
	<b>PLA</b>	<b>CSR</b>	<b>PA</b>	<b>PP</b>
<b>PLA</b>	100	0	0	0
<b>PLAPA</b>	99	0	1	0
<b>PLAPACSR1</b>	98	1	1	0
<b>PLAPACSR3</b>	96	3	1	0
<b>PLAPACSR5</b>	94	5	1	0
<b>PLAPACSR7</b>	92	7	1	0
<b>PLAPACSR10</b>	89	10	1	0
<b>PP</b>	0	0	0	100

## 2.2. Preparation of the modified PLA

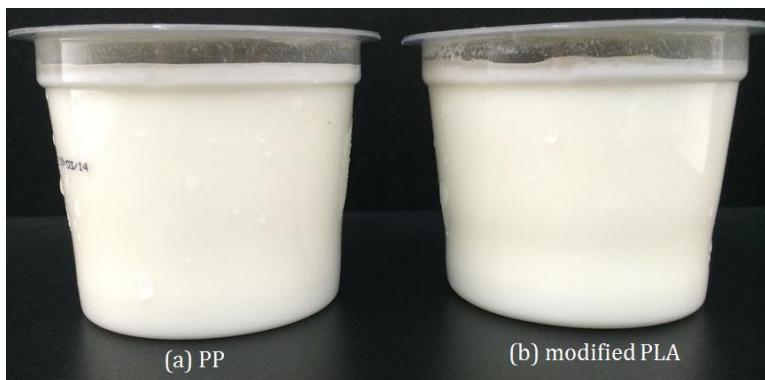
The preparation of the impact modified PLA in the present study consisted of 3 experimental parts. The first was the dry-blending of PLA with 1wt% PA and various CSR contents of 1.0, 3.0, 5.0, 7.0 and 10wt%. The PLA/CSR/PA compositions were then melt-blended and pelletized by using a co-rotating twin screw extruder (Labtech, LTE 20-40) and a pelletizer. The screw speed was set constantly at 100 rpm while the barrel temperature was ranged from 155 to 185°C. Finally, they were injected by using an injection molding machine (Manumold) for subsequent the mechanical tests. The injection conditions were clearly indicated in Table 2.

**Table 2. Injection molding conditions.**

Designations	Value
Barrel temperature Zone1/Zone2/Zone3	190/195/200 °C
Injection feed forward I pressure	40-80 bar
Injection feed forward II pressure	40-80 bar
Injection feed forward III pressure	40-80 bar
Holding pressure	20 bar
Mold clamping pressure	60 bar
Ejector pressure	40 bar
Mold temperature	45°C

## 2.3. Fabrication of modified PLA and PP yogurt cups

After selecting a desired PLA/CSR/PA composition from the mechanical test results in Section 2.2, PLA CSR and PA were compounded by using an industrial-scaled twin screw extruder and then pelletized to obtain the PLA compound pellets. PLA compound and neat PP were then sheeted and thermoformed by using an industrial-scaled sheet extruder equipped with chill rolls (Meaf, Sheetline 75-H34P) and thermoforming machine (Cheng Met, CM F1000). The average thickness of the sheet and the thermoformed yogurt cup was 450 and 250 µm respectively.



**Fig. 1. Visual appearance of set yogurt stored in (a) PP and (b) modified PLA packages.**

## **2.4. Preparation of set yogurt and storage condition**

The skimmed milk was inoculated with yogurt culture and incubated. After pre-cooling at ambient temperature, the yogurt sample was filled in both the modified-PLA and PP cups prior to seal with aluminium foil as illustrated in Fig. 1. The yogurt was then incubated at 5°C for 18 days to observe the quality changes.

## **2.5. Testing and characterizations**

### **2.5.1. Testing and characterizations of modified PLA and PP**

A notched Izod impact test was evaluated by using an impact tester (Yasuda, 190) in order to determine the energy required to break the test specimen upon impact force following the procedure described in ASTM D 256. The oxygen transmission rate (OTR) was evaluated in accord with ASTM D3985 by using a gas permeation tester (Mocon OX-TRAN, 2/21). The temperature and relative humidity (RH) of the test conditions were 25°C and 0%RH respectively. The samples were conditioned for 5 hours prior to test. The OTR determination was performed until 10 values of the constant transmission rate were obtained. The final transmission rate was averaged over the last 10 constant values for the replicate samples. The melt flow index (MFI) was evaluated following the process described by ASTM D 1238. The temperature and the piston load were set at 190°C and 2.16kg respectively.

### **2.5.2. Testing of the food stimulant**

Color measurement was carried out by using a Hunter Lab Colurflex colorimeter. The colorimeter was adjusted for reflectance, illuminant D 65, and angle of 10°. Color measurement data was provided in accord with the CIE system in terms of  $L^*$  (lightness),  $a^*$  (redness and greenness) and  $b^*$  (yellowness and blueness).

The textural hardness measurement was applied on set yogurt at room temperature by using a universal testing machine (Instron, 5966) equipped with a 1cm diameter stainless steel probe. It was employed to evaluate the hardness of the set yogurt during storage in two different packaging materials. The cross-head speed and the compression distance were set at 5 mm/min and 2.5 mm respectively. A maximum load of ten replicate samples were averaged and reported as the hardness.

The syneresis value of the yogurt was evaluated following the procedure described by Keogh and O'Kennedy [14]. Twenty five grams of set yogurt were prepared in a centrifuge cup and then centrifuged at 2500 rpm for 10 min at room temperature. The transparent phase was then collected and measured. The syneresis value was calculated following Eq. (1):

$$\text{Syneresis (\%)} = \frac{\text{Weight of transparent phase}}{\text{Weight of yogurt sample}} \times 100 \quad (1)$$

The acidity was estimated following the process described by Mistry and Hassan [15]. The pH was determined by using a digital pH meter (Mettler Toledo,

FiveEasy<sup>TM</sup> pH). The lactic acid bacteria count was analyzed using the pour plate technique on MRS agar and the plates were incubated at 37°C for 2 days.

### 2.5.3. Biodegradability of packaging materials

The biodegradability of yogurt cup was estimated by measuring the carbon dioxide content following the procedures described in ISO 14855 using a Microbial Oxidative Degradation Analyzer (Saida FDS, MODA 6).

### 2.5.4. Statistical analysis

The experimental design was a completely randomized design (CRD). Data was subjected to analysis of variance (ANOVA). The comparison of means was carried out by Duncan's multiple-range test.

**Table 3. Mechanical properties of PP, PLA and modified PLA.**

Formulation code	Impact strength (J/m)	MFI at 190°C (g/10 min)	OTR (ml/m <sup>2</sup> .24days)
<b>PLA</b>	24.1±0.12 <sup>h</sup>	6.9±0.47 <sup>a</sup>	58.2±0.64 <sup>c</sup>
<b>PLAPA</b>	25.7±0.19 <sup>g</sup>	2.1±0.60 <sup>d</sup>	n/a
<b>PLAPACSR1</b>	31.6±0.89 <sup>f</sup>	2.4±1.31 <sup>d</sup>	n/a
<b>PLAPACSR3</b>	57.0±0.60 <sup>e</sup>	2.3±0.05 <sup>d</sup>	n/a
<b>PLAPACSR5</b>	98.6±0.06 <sup>c</sup>	2.9±1.50 <sup>d</sup>	64.6±0.13 <sup>b</sup>
<b>PLAPACSR7</b>	114.0±2.89 <sup>b</sup>	4.3±0.43 <sup>c</sup>	n/a
<b>PLAPACSR10</b>	129.1±1.33 <sup>a</sup>	5.6±1.58 <sup>b</sup>	n/a
<b>PP</b>	97.6±0.47 <sup>d</sup>	2.7±0.21 <sup>d</sup>	1975±5.96 <sup>a</sup>

For each column, means with the same letter do not differ significantly at p≤0.05

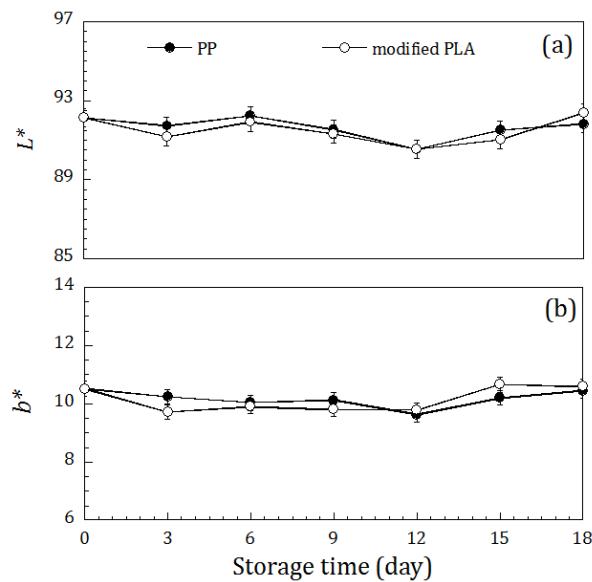
n/a = no data available

## 3. Results and Discussions

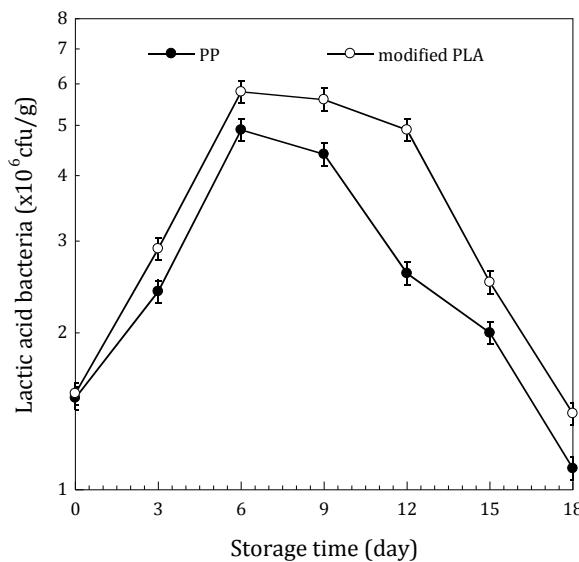
### 3.1. Physical and mechanical properties of packaging materials

It is widely known that the problems of brittleness and low impact resistance have limited the use of PLA in many applications especially for food packaging [1, 8-10]. A notched Izod impact test was employed to determine the impact resistance of neat PLA and modified PLA and compared with that PP. With the presence of 1wt% PA, PLA showed slightly higher impact strength while the MFI was dropped by more than threefold approaching the MFI value of PP. As shown in Table 3, a substantial increment in the impact strength was observed in all PLA/CSR/PA compositions. Adding 5wt% CSR to PLA/PA increased the impact resistance higher than that observed in neat PLA and PP. This indicated that CSR particles could absorb larger amounts of energy upon the impact force [4] than that was observed in the neat PLA and PP. Numerous studies have reported a drastic increment in the impact strength when an acrylic based toughening agent was added to PLA. Petchwattana *et al.* [4] found a rise in the impact strength by four times when AR was added to PLA at 10wt%. Oyama [16] reported a fourfold increment in the impact strength when the poly(ethylene-glycidyl methacrylate)

(EGMA) was added to PLA. In term of mechanical performance and processability, the PLA/CSR/PA blend could be used as a yogurt package as good as the PP could.



**Fig. 2. Change in lightness ( $L^*$ ) and yellowness ( $b^*$ ) of set yogurt during storage in different packaging material (a)  $L^*$  and (b)  $b^*$ .**



**Fig. 3. Viability of lactic acid bacteria in set yogurt during storage in two different packaging material.**

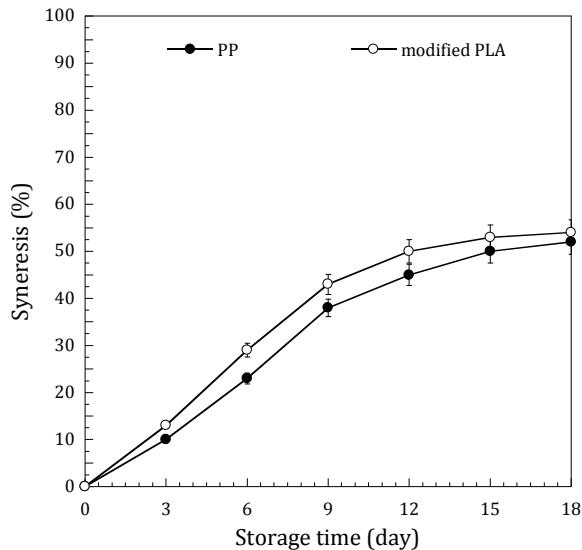


Fig. 4. Syneresis of set yogurt during storage in different packaging material.

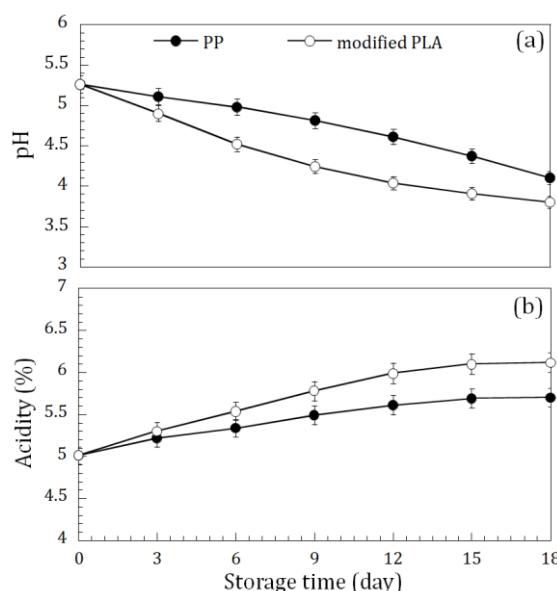
### 3.2. Changes in properties of set yogurt

Fig. 2 shows the color measurements of yogurt samples with various storage time and packaging materials. The yogurts stored in both PP and modified PLA cups showed undetectable difference in terms of lightness and yellowness. The  $L^*$  and  $b^*$  values were observed at around 92 and 10 respectively. These values indicated that the set yogurts were originally white and light yellow. Types of packaging material and storage time did not show significant ( $P>0.05$ ) effect on the lightness and yellowness of the yogurt.

Changes in the number of viable lactic acid bacteria over the storage period were also investigated and graphically presented in Fig. 3. The bacterial amounts were influenced by the packaging material as well as storage time. Initially, the total lactic acid bacteria in the yogurt were  $1.50 \times 10^6$  and  $1.53 \times 10^6$  cfu/g for the PP and modified PLA packages respectively. After storage at  $5^\circ\text{C}$  for 6 days, the number of bacteria grew significantly ( $P<0.05$ ) with time and reached their maximum values at  $4.4 \times 10^6$  and  $5.6 \times 10^6$  cfu/g for the PP and modified PLA respectively. Beyond 6 days, the counts decreased dramatically due to the increased lactic acid concentration and the decreased pH which directly reduced the survival of the bacteria. The number of bacteria declined after being stored for 18 days by around  $1.1 \times 10^6$  and  $1.4 \times 10^6$  cfu/g for the PP and PLA packages respectively. These values were larger than the minimum amount of bacteria required for the health benefits [17]. In comparison, the modified PLA exhibited higher bacterial counts than PP at all durations of storage. This is due to the fact that lactic acid bacteria were categorized as facultative bacteria which required less oxygen for survival. Higher OTR of PP (see Table 3) was the cause of their viability and growth restrictions under the excess oxygen environment. This could be preliminary summarize that the yogurt stored in modified PLA package gave more health benefits than PP package. In milk, Klaver and others [18] also found

a negative impact on the viability of bacteria such as *Lactobacillus* and *Bifidobacterium spp.* under a high concentration of oxygen. Another investigation compared the survival of bacteria in yogurt stored in polystyrene (PS) and glass bottles. The authors reported that the yogurt contained in glass bottle had more survival of *L. acidophilus* than that stored in PS over 35 days [19].

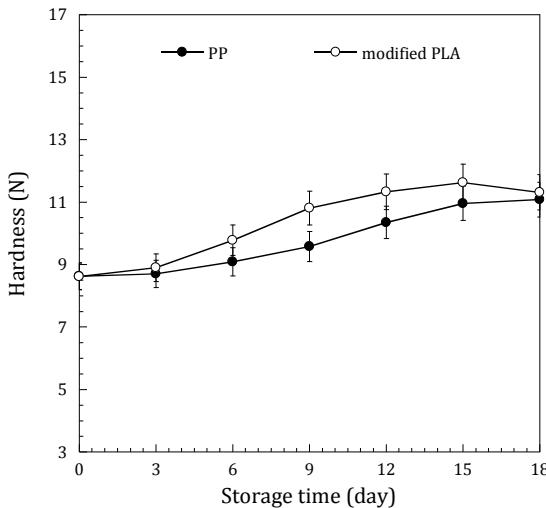
Syneresis is generally recognized as the shrinkage of the gel bringing the liquid separation from the yogurt curd, which is an undesired property. It is directly correlated to the acidity and is inversely related to the pH [20]. Fig. 4 shows the changes in the syneresis of set yogurt during incubation. As expected, the degree of syneresis increased at all yogurt samples and all storage times. This rise can be separated in two periods. Earlier than day 9, the syneresis increased at very rapid rate leading the aggregation of casein micelles. After this period, the syneresis still increased but at much lower rate. Generally, syneresis of yogurt occurs when the use of high incubation temperature, excessive whey protein/casein ratio, low solid content and physical mishandling during storage [21]. In this case, higher lactic acid bacteria levels tended to increase the hydrogen ion concentration during acidification and were the cause of casein micelles aggregation. This made set yogurt stored in modified PLA package had higher syneresis value than PP package.



**Fig. 5. Change in chemical properties of set yogurt during storage in different packaging material (a) pH and (b) acidity.**

Generally, the pH of the set yogurt is directly affected by the lactic acid bacterial activity and storage time. Fig. 5 shows the pH and the acidity of set yogurt during storage. The initial pH of set yogurt was 5.26 and 5.24 for modified PLA and PP packages respectively. These values were dropped significantly ( $P<0.05$ ) by storage time. This can be explained by the fact that lactic acid was

produced by bacteria during storage. This inversely related to the increased acidity as illustrated in Fig. 5(b). Of the two packaging materials, modified PLA package was found to have lower pH than that observed in PP package. This was due to the higher production rate of lactic acid derived from the higher bacterial counts as previously discussed.



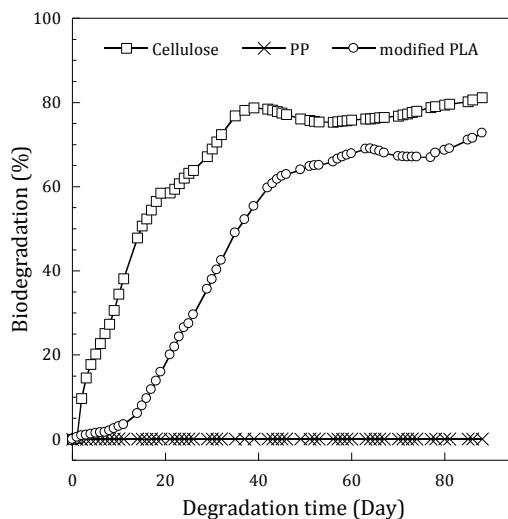
**Fig. 6. Hardness of set yogurt during storage in different packaging material.**

Hardness is one of the parameters that affect the sensorial perceptions of consumers [22]. Fig. 6 illustrates the relationship between the hardness and the storage time of the yogurt stored in the different packaging materials. The hardness of the set yogurt within the PP and modified PLA packages at the day 0 was 8.63 and 8.62 respectively. Set yogurts kept in both packaging material showed significant increment in the hardness during storage period ( $P<0.05$ ). This hardening of yogurt gels could be explained through protein aggregates being formed by the interaction between the denatured whey proteins and the casein micelles via intermolecular disulfide bonds [22-23]. Compared to PP package, the modified PLA package showed a greater hardness which occurred beyond day 3 due to the significant increment in syneresis as previously discussed in Fig. 4.

### 3.3. Biodegradability of packaging materials

As illustrated in Fig. 7, the biodegradability of each packaging material was evaluated by collecting the amount of  $\text{CO}_2$  compared to cellulose samples. Of the materials studied, cellulose started to degrade earlier than modified PLA and PP packages. Cellulose reached its maximum biodegradation of around 80% within 40 days which was valid the reference material defined by ISO 14855. The modified PLA package started to degrade at a very slow rate around the first 10 days. Beyond this period, the modified PLA package tended to degrade at a faster rate and achieved approximately 50% biodegradation at around 40 days. The

modified PLA package continued to degrade and reached a degradation plateau at 60 days while the PP package did not show any biodegradation. The maximum degree of biodegradation of the modified PLA was around 70%. This clearly confirmed the biodegradability of the modified PLA package. A similar display of biodegradation was observed in the PLA/starch blends. Petinakis *et al.* [24] found that the rate of biodegradation for the PLA/starch blends (more than 80%) was higher compared to that of pure PLA. Liu *et al.* [25] indicated that there was a higher degradation rate of PLA package after incorporating sugar beet pulp. From the economic point of view, the modified PLA package is more expensive than PP packages by around 40%. This limitation still limited the use of PLA for the commodity products such as yogurt.



**Fig. 7. Biodegradation of PP and modified PLA packages compared to cellulose.**

#### 4. Conclusions

Adding 5wt% CSR gave PLA/PA as tough as PP. Types of packaging material and storage time did not change the color of set yogurt. The number of lactic acid bacteria grew significantly after they had been incubated for 6 days. After that, the bacterial viability was decreased dramatically due to the increased acidity and the decreased pH. A positive impact on the viability of bacterial growth was found when set yogurt was stored in modified PLA package. This made the yogurt had more health benefits than stored in PP package. The biodegradation test results indicated that the modified PLA package degraded at a rapid rate. It achieved approximately 50% biodegradation at around 40 days which was comparable to cellulose whereas PP package was non-biodegradable. In summary, substitution conventional PP package by a novel modified PLA package seems to be a better way for both the health and the environment benefits.

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