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School of Packaging, Michigan State University

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Development of Model Active Packaging System and Inactivation of Surface-Associated *Listeria Monocytogenes* by Controlled Release of Nisin

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ABSTRACT: We introduce a simple model food/packaging system to study the efficacy of controlled antimicrobial agent (AMA) release on the bacterial growth inhibition. The system permits considering the effect of headspace, either liquid- or gas-filled. Both types of headspace showed significant decrease in the bacterial growth inhibition due to limited AMA transport from the packaging layer to the model food. The model food/packaging system has been validated on a ready-to-eat meat product (sliced turkey), and reasonable bacteria inhibition levels were achieved.

THE food market has growing demand for fresh and minimally processed foods. However, these foods are highly perishable and more susceptible to microbial spoilage. Thus, there is a strong need to develop new preservation methods to achieve a required level of safety, quality and nutritional value of food during extended shelf life period. The use of active packaging (AP) materials is one of the post-processing methods to preserve food products and meet consumers' expectations.

Antimicrobial packaging is designed to control microbial growth in a food product. It consists of an antimicrobial agent (AMA) immobilized onto internal surface of a package or incorporated into packaging material (Han and Floros, 1998). In the latter case AMA is released into a food product over time. This permits to extend shelf life of food products, helping to reduce the amount of AMA in food formulation.

AP materials have many parameters that influence their antimicrobial efficacy, which is thoroughly addressed in a number of studies: polymer processing, polymer morphology (Petersen et al., 1999), polymer swelling (Buonocore et al.) and AMA affinity to the packaging material (Soliva-Fortuny and Martin-Belloso, 2003). The food product was considered in all these papers as a homogeneous medium in full contact with the packaging. However, surface morphology of the foods is an important parameter that determines mass transfer of an antimicrobial agent through the interface between packaging and food.

VARIOUS TYPES OF FOOD/PACKAGING CONTACTS

Based on the nature of a food product and corresponding morphology of a food surface one can distinguish five types of food-packaging contacts depicted in Figure 1.

If the surface of a food product is flat, direct contact between the packaging and the food exists. This AP system has maximum efficacy. An irregular food surface will cause only partial contact between the packaging material and the food product, developing non-continuous headspace. The headspace configuration influences AMA transport from the packaging to the food. Depending on the dominating physical state of a food product, the packaging surface can be in contact with solid or with liquid food products, or sometimes both. These contacts can be direct or indirect if headspace exists between the food surface and packaging material (see Figure 2). Depending on the type of the food product, the headspace can be liquid- or gas-filled.

Numerous parameters can influence the efficacy of AP, including the packaging material properties, the antimicrobial transport, the bacterial population response and the food matrix. Most of the published studies investigated antimicrobial activity of the controlled release compound by adding AMA directly to the foods

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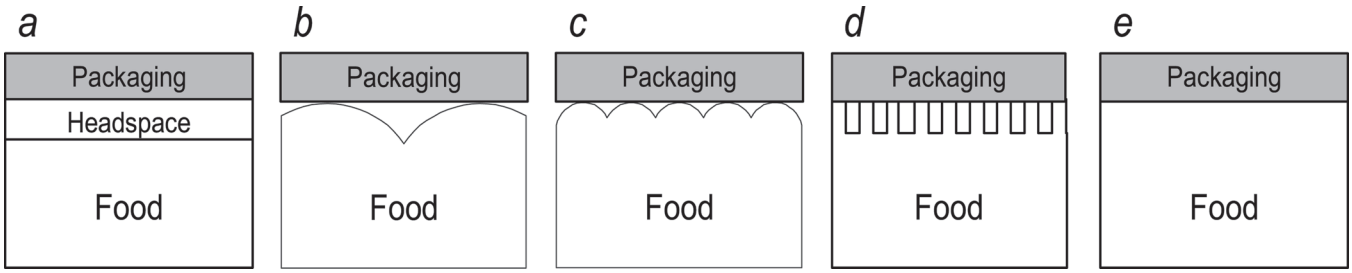

Figure 1. *Spatial organization of AP as a function of food surface morphology: direct contact (a), partial contact ~1 cm (b), ~1 mm (c), ~10 μm (d) and direct contact (e).*

or to the packaging materials. However, these two methods have significant disadvantages:

- When an AMA is added directly to the food, experimental data provide will important information on the antimicrobial activity of AMA and its interaction with the food matrix. There is no time-dependent AMA release; therefore these studies are insufficient for the development of AP.
- On the other hand, if AMA is incorporated into the packaging material, there is no control over the antimicrobial release. The effects of packaging material properties on the AMA release rate cannot be distinguished from the effects of the AMA release rate on the bacterial inhibition.

There is a need to understand bacterial response to the AMA release without the influence of material-dependent properties of AP. No standard method has been established to investigate the effect of antimicrobial agent's time-dependent release on the bacterial response. This study aims to design a method that will link microbiological studies to packaging design by developing a model food/packaging system with controllable release properties and flexible configuration.

MATERIALS

Model Microorganism

Listeria monocytogenes strain 10403, a human clinical isolate, serotype 1/2a (purchased from Dr. Portnoy, University of California, Berkeley) was used to test the efficacy of AP model. The stock culture was maintained at –80°C in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) with 20% Glycerol. The cells were stored on BHI agar (Difco) at 4°C and sub-cultured every two weeks in BHI broth at 30°C for 20 hours. Before each experiment the cells were brought to mid-log phase by adding 1 ml of the subculture to 9 ml of BHI broth and incubating at 30°C for 4 hours.

Antimicrobial Agent

Nisin—the bacteriocin produced by *Lactococcus lactis*—has been shown to be effective against *L. monocytogenes.* Nisin creates pores in the membranes of Gramm-positive bacteria and causes the dissipation of transmembrane potential, creating collapse of the proton motive force and the lysis of the cell (Szabo and

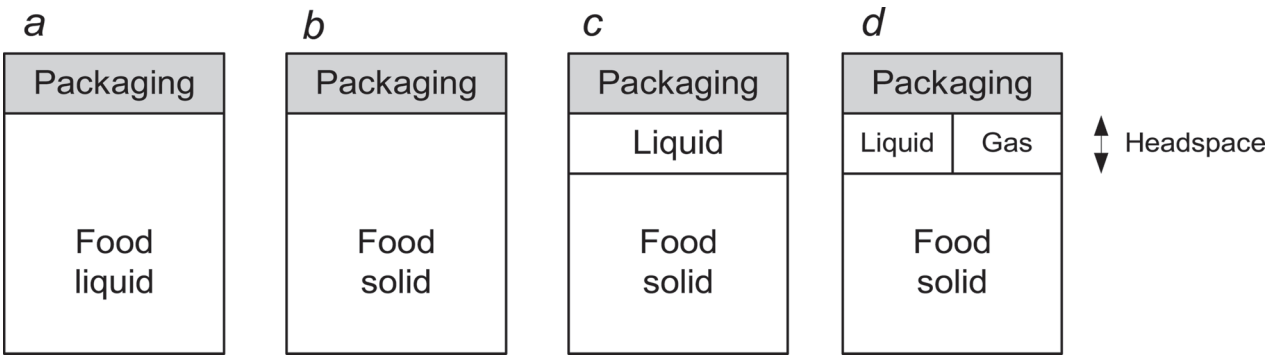

Figure 2. *Dependence of a food/packaging interface on a type of food product.*

Cahill, ; Thomas et al., 2002). It is the only bacteriocin generally recognized as safe in the US for use in processed cheese spread and it is approved for use in selected foods in more than forty countries (Abee and Wouters, 1999). Commercial-grade nisin (nisalpin) purchased from Sigma Chemical Co. (St. Louis, MO) was dissolved in sterile water and adjusted to pH 2 with hydrochloric acid. A fresh solution of 10,000 IU/ml was prepared before each experiment.

Determination of Nisin Activity

L. monocytogenes was inoculated to get an initial cell concentration 10^5 cfu/ml. Immediately after inoculation the samples were incubated at 30°C and the growth was assessed over 48 hours by absorbance measurements using the MRX II microplate reader (Dynex Technologies, Chantilly, VA).

After 24 hours exposure to different nisin concentrations 20 µl of each culture were re-suspended in 10 ml fresh BHI broth with no nisin. The samples were incubated at 30°C and the growth was assessed over 24 hours by optical density reading using the microplate reader.

Model Semi-Solid Food Product

Since most of listeriosis outbreaks are associated with the surface contamination of ready-to-eat meat products, our food model should represent this type of surface. The food model selected for this system was Tryptic Soy Agar (TSA, Difco), because it has been shown to be a good surrogate of meat surface (Midelet and Carpentier, 2002). Prepared TSA has a final pH of 7.3, and an agar concentration of 15 g/L.

Tested Food Product

Sliced lean white turkey, oven-roasted style (Butterball, ConAgra Foods, Omaha, NE) was used in this research; its pH = 6. To quantify bacterial load, a total plate count (TPC) assay was performed by stomaching 25 g of sliced turkey in 225 ml of 0.1% peptone water for 1.5 minutes, and plating dilution 10° to 10^{-4} on TPC agar (Difco). No growth has been observed after incubation of the TPC plates at 30°C for 48 hours.

Model Material for Active Packaging

Agar is a porous solid matrix that permits easily control the antimicrobial release rate. The diffusion coefficient of nisin into 3% agarose has been determined by (Sebti et al., 2004). It has been estimated to be a 8.14×10^{-11} m^2/s. They have also shown that the nisin concentration in gel does not influence the diffusion process. Nisin diffusivity in agar remains constant until agarose concentration reaches 8%.

Agar (Difco) was used as an entrapment matrix for nisin. Agar was dissolved to obtain a 2% solution and autoclaved at 121°C for 15 minutes. Prepared agar was poured into Petri dish to form a layer. Changing the amount of agar one can obtain model packaging materials with various thicknesses.

Evaluating the Effect of AMA Load on Bacterial Growth

To evaluate the influence of antimicrobial agent load five model packaging layers with predetermined concentrations of nisin (0, 10, 100, 500, and 1000 IU/ml) were prepared. *L. monocytogenes* culture was inoculated on the surface of TSA plates to obtain overall concentration of 100–300 cfu/plate. The agar "packaging" layers were placed on top of inoculated TSA surfaces [see Figure 3(a)]. The resulting system consists of surface-contaminated meat surrogate (TSA) and controlled-release (active packaging) material in direct contact with contaminated food.

Evaluating the Effect of Air Filled Headspace on the Efficacy of AMA Release

To investigate the effect of air filled headspace, samples were prepared with inoculated TSA and nisin layers of 0, 10, 100 and 1000 IU/ml. Air gaps were created at the periphery of the plates, so there was direct contact between nisin-containing layer and the "food" layer at the center of the plate, and the air-filled headspace surrounding the direct contact area [see Figure 3(b)]. The plates were incubated at 30°C for 36 hours; bacterial growth was assessed by direct colony count in the areas of direct contact and in the regions with the air-filled headspace.

Evaluating the Effect of Liquid Filled Headspace on the Efficacy of AMA Release

For the experiments with liquid-filled headspace both TSA and turkey slices were used as target food surfaces. The AMA loads in the nisin layer were 0, 100 and

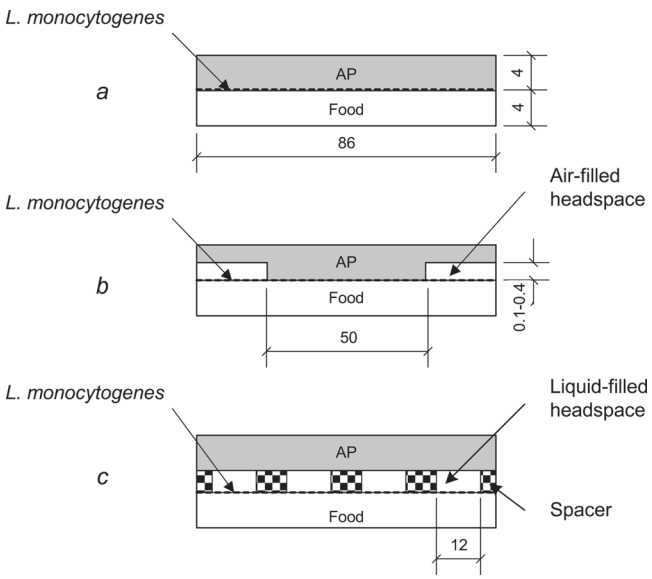

Figure 3. *Food packaging model system (all dimensions in mm).*

1000 IU/ml. To create the headspace we have made a spacer from nylon mesh and placed it between the model food and "packaging" layer [see Figure 3(c)]. To mimic headspace liquid, 8 ml of 0.1% peptone water was poured over the food surface. Samples were incubated at 30°C for 36 hours; stomached and plated on Modified Oxford medium. Direct colony count was performed after appropriate incubation period as described above.

RESULTS

Design of the Model Food/Active Packaging System

The first objective of the study is to design a system that will deliver an AMA with continuous release. The packaging material should not be subject to swelling. It should be favorable to bacterial growth (by not adding supplementary stress), but it should not enhance the growth by, for example, providing extra nutrients. The properties of the designed model system will be similar to AP, but the effect of the packaging material matrix on the release process will be controllable.

A model food/packaging system was designed in the way to obtain homogeneous distribution of AMA within the packaging matrix, and insure controllable release of the stored antimicrobial to the food. We utilize a sandwich-type design of the model system, see Figure 3. The bottom layer is the food product (model or real) with surface inoculated by *L. monocytogenes*. The top layer consists of agar with entrapped antimicrobial agent molecules. Agar layer used as the model packaging matrix has three parameters that can be used to control nisin release rate: viscosity, thickness, and AMA load.

Diffusion of Nisin from Plane Surface AP into Semi-Infinite Food Medium

Let us consider a food/packaging system as depicted in Figure 3. AMA diffuses through the polymer matrix towards the interface between packaging and food. This process is driven by concentration difference between the packaging and the food product. In case of amorphous polymer matrix above its glass transition temperature diffusion mechanism of an AMA can be described by Fick's law, i.e. AMA concentration change with time is proportional to the rate of antimicrobial agent concentration gradient change with distance:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{1}$$

A common assumption in the packaging literature is that the diffusion is unidirectional and diffusion coefficient is constant within each material layer (packaging, headspace, and food). The diffusion rate is determined by the properties of the polymer matrix, interaction between AMA and packaging material (solubility of the AMA in the polymer and interaction forces between the AMA and polymer molecules), and environmental factors: temperature, pressure and the composition of the food matrix. The antimicrobial agent migrates from the package across the interface between the polymer matrix and the food. When packaging material is not in the direct contact with the food product, AMA migration through this headspace can be a rate-limiting step for the whole delivery process.

It is clear that the diffusivity of an antimicrobial agent wouldn't be a rate-limiting step for AMA release, since the viscosities of the food product and packaging material are much higher than that for the headspace-filling substance. Even more, if headspace is thin enough it will not affect the overall delivery rate of the released agent. In this case the migration time of antimicrobial compound through the headspace is much smaller than the characteristic migration (diffusion) times for the packaging material and food product, and obviously, the shelf-life period. The last requirement is important

for large food products ($L_f \approx l_p$, where L_f, l_p are the characteristic size of a food product and the thickness of an AP material correspondingly), that can be considered as semi-infinite media. It is well known that the characteristic diffusion time scale is $\sim l^2/D$. Therefore, "no headspace impact" condition can be written as:

$$\delta \approx \min\left[l_p \sqrt{\frac{D_\delta}{D_p}}; \sqrt{D_\delta t_{sl}}; L_f \sqrt{\frac{D_\delta}{D_f}} \right] \qquad (2)$$

where D_δ, D_p, D_f are the diffusivities of AMA in the headspace medium, packaging and food respectively; δ—the headspace thickness; t_{sl}—the shelf-life period or total time of experiment.

The typical diffusion coefficient for packaging materials is about $10^{-12} \ldots 10^{-15}$ m^2/s, and the thickness is ~100 … 400 μm. Limiting condition (2) is valid for liquid-filled headspaces with thicknesses up to ~500 μm. However, for gas-filled headspaces requirements (2) are difficult to satisfy due to the low mobility of AMA in the gas phase.

If a liquid-filled headspace in a food/packaging system satisfies (2) it can be excluded from the model, since it does not impact diffusion-controlled transport of released antimicrobial agent. This food/packaging system is analogous to that depicted in Figure 1(e). It can be represented as a semi-infinite medium, i.e. a homogeneous food product with a thin surface layer where the transport properties differ from those of the rest of the medium. The boundary conditions at the food/packaging interface ($x = 0$) are continuity and "no accumulation":

$$\begin{aligned} C_p &= C_f \\ D_p \frac{\partial C_p}{\partial x} &= D_f \frac{\partial C_f}{\partial x} \end{aligned} \qquad (3)$$

Following the method described in (Crank, 1975), the solution of Equation (1) with boundary conditions at the food/packaging interface, zero initial concentration of AMA in the food, and initial concentration of antimicrobial agent C_{p0} loaded into the packaging material can be written in the form:

$$C^*(t) = \frac{1}{2} \operatorname{erfc} \frac{1}{2\sqrt{\mathrm{Fo}}} \qquad (4)$$

where,

$$C^*(t) = \frac{C_f(t)\big|_{x=0}}{C_{p0}}$$

and

$$F_o = \frac{tD_p}{l_p}$$

is the Fourier number of a packaging material.

One should note that there is a difference between the model gel-type AP material used in our experiments and a real food/packaging system. The model packaging material and food product studied both have similar transport properties ($D_p \sim D_f$), while for the majority of "real life" packaging materials these properties are quite different $D_p \sim D_f$. We can use the solution (4) to analyze the model food/packaging system in terms of controlled release of the active compound. (Sebti et al., 2004) studied nisin diffusion in 3% agarose gel. The diffusion coefficients at 5.4 and 22.3°C were found to be equal to 1.92 and 8.14 × 10^{-11} m^2.s^{-1} respectively. The diffusion coefficients in agar can be assumed to be of the same order.

It is clear that for surface-contaminated food products homogeneous active packaging material provides a bi-modal AMA delivery. For the initial period of time (~12 hr) the AMA surface concentration is almost constant and, therefore, its action is similar to the formulation-based AMA delivery. For extended shelf-life period the concentration of AMA exponentially decreases. Therefore, the microbial population responses to the contact with active packaging material significantly differ for long- and short-time shelf life periods.

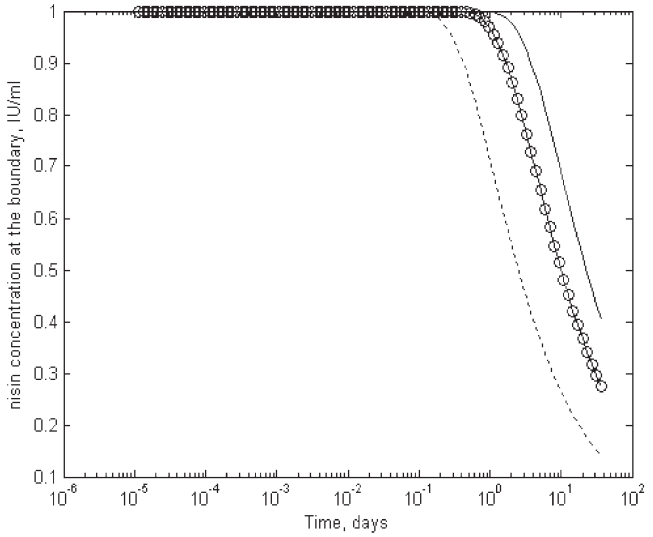

Figure 4. *Nisin concentrations at the agar surface with initial nisin concentration of 100 IU/ml and for the agar layer thicknesses: — 2 mm, ○ 4 mm, and - - 6 mm.*

Direct Contact Mode: Concentration of AMA Incorporated into the Agar Matrix Affects Bacterial Growth

Once the system was designed, the first step was to vary one of the parameters: the concentration of nisin incorporated into the packaging matrix to obtain inactivation kinetics with different nisin release profiles and compare the results with the standard method. When the nisin concentration in agar layer is high, the AMA release rate should increase influencing bacterial inhibition. We have used relatively small concentrations of nisin because of the consumer demand for food products with minimal amount of additives. Additionally, all active packaging materials can be characterized by very high retention rates with the total amount of released antimicrobial not exceeding 5% of its actual load. All samples in this experiment were made by inoculation of TSA surface by *L. monocytogenes* 10^2 cfu/plate with following incubation for 36 hours at 30°C. The only variable in this experiment was the nisin concentration that was varied from 0 to 1000 IU/ml.

To separate the effect of the model packaging material (agar) and AMA we have performed a control experiment comparing TPC values for inoculated TSA plates uncovered and covered with agar layer with no nisin added. The "food" sample covered with agar layer had bacterial counts 190 cfu/dm^2, while uncovered "food" sample had 230 cfu/dm^2, which is a normal error for plate counting method. Hence, agar "packaging" layer does not affect bacterial growth. All observed changes in bacterial growth are, therefore, due to presence of nisin in the packaging layer.

One should note that bacterial colonies developed under the agar layer were much larger than the colonies on the surface without agar. This can be explained by lower oxygen availability under the agar layer. (Nilsson et al., 1997) showed that *L. monocytogenes* growing under 100% CO_2 atmosphere had 2–5 times more elongated cells. This suggests that the changes in the cell morphology can be responsible for the shape of colonies that are formed under limited oxygen supply.

Figure 5 shows data on bacterial survival obtained in the agar packaging layers with various nisin concentrations. The layer containing 10 IU/ml of nisin provides 40% inhibition compared to the 0 IU/ml control sample. The 100, 500 and 1000 IU/ml samples showed significant (100-fold) bacterial inhibition. The maximum inhibition effect was observed for 500 IU/ml.

The results observed are consistent with the operational mode of the system designed. As the AMA load increases in the "packaging" layer, the nisin release rate also increases due to higher concentration gradient across the food-packaging interface. The increased release rate enhances microbial inactivation. The relatively low effect of packaging layer with low nisin content can be explained by *L. monocytogenes* tolerance of nisin and the existence of sublethal bacteriocin dose.

The 10 IU/ml nisin load had little effect on the bacterial growth, but concentrations above 100 IU/ml had considerable effect. The bacterial growth observed at

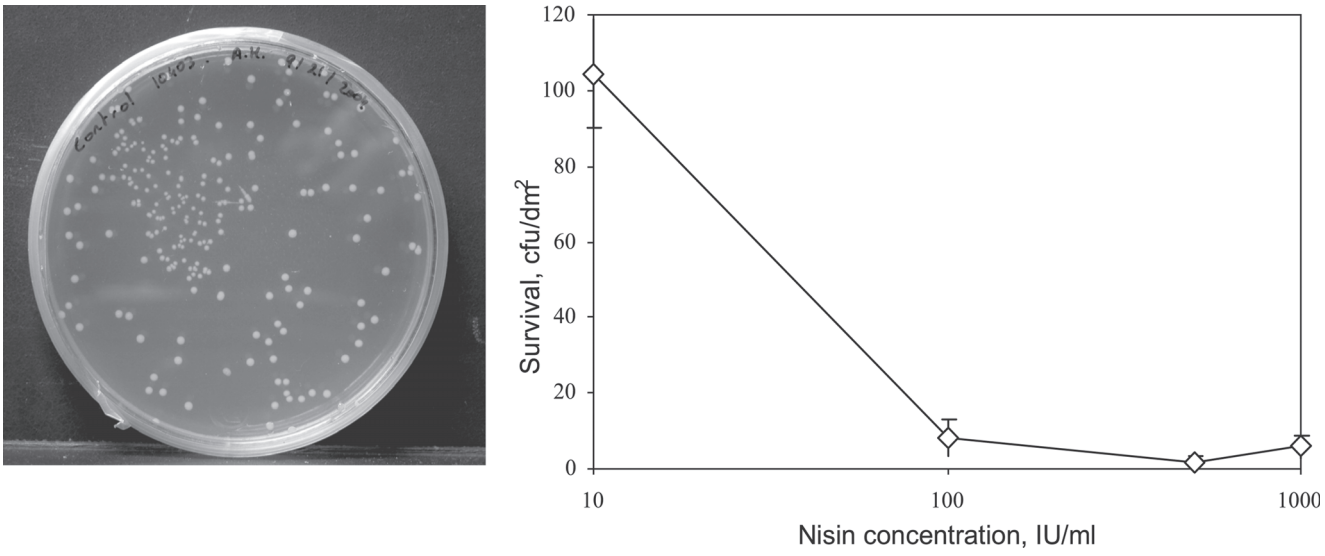

Figure 5. *Inhibition of* L. monocytogenes *under AP layer in direct contact with the model food: a)* Listeria *colonies under agar layer containing 100 IU/ml; b) bacterial survival as a function of nisin load of the AP layer.*

the nisin load of 1000 IU/ml could be explained by either stress adaptation or nisin-resistant bacterial mutants development, as described by (Chi-Zhang et al., 2004).

Effect of Air Filled Headspace on the Efficacy of Antimicrobial Controlled Release

The efficacy of antimicrobial packaging is affected by the presence of an air-filled headspace between the food surface and the package. It is often observed for irregularly-shaped foodstuff (e.g. vegetables, meet, etc.). The food/packaging model developed allows for investigation of the headspace effect.

We have designed the experimental setup so that a "ring of air" (i.e. gas-filled headspace) has been formed within the Petri dish along its perimeter [see Figure 6(a)]. Therefore, our model system contained two distinct regions with different AMA release conditions: a "direct contact" food/packaging zone with the agar layer in the center of the plate, and a "non-contact" zone at the periphery. The width of the air-filled "ring" (a) has been chosen to be $a = R(1-1/\sqrt{2})$, so the areas of direct contact zone and air-filled headspace were equal. Samples were prepared with 0, 10, 100, 500, and 1000 IU/ml loads of nisin.

The control sample with the agar layer containing no nisin had 190 cfu/dm^2 in the area of direct contact zone, and 118 cfu/dm^2 under the air-filled headspace. The results depicted in Figure 6(b) show that there was no significant inhibition of bacterial growth under the air filled headspace. Thus, the antimicrobial efficacy of the packaging is significantly reduced by the presence of gas-filled headspace due to low gas mobility of antimicrobials. Accordingly to Graham's law, molecular mobility of substances in gases is:

$$D \propto \frac{1}{\sqrt{MW}} \tag{5}$$

where MW is the molecular weight of the substance. Since the molecular weight of nisin is 3354.07, its mobility in the air is ~10.6. times lower than that of oxygen.

Liquid-Filled Headspace and the Efficacy of the Active Packaging

Many packaged food products contain a liquid-filled headspace. This headspace can exist in two cases: juice/liquid naturally extracted from the product as a result of its processing or storage; and liquid added to the product for food preservation and/or conditioning. The presence of liquid in the headspace could limit the transport of AMA from the package to the food.

The sliced turkey food sample was used for this experiment. The headspace was created by a spacer made of nylon mesh, filled with peptone water. The agar layer had nisin concentrations of 0, 100, or 1000 IU/ml. Bacterial growth levels were measured after 36 hours of incubation at 30ºC.

The results are displayed in Figure 7. As it was expected, the samples with headspace had higher levels of

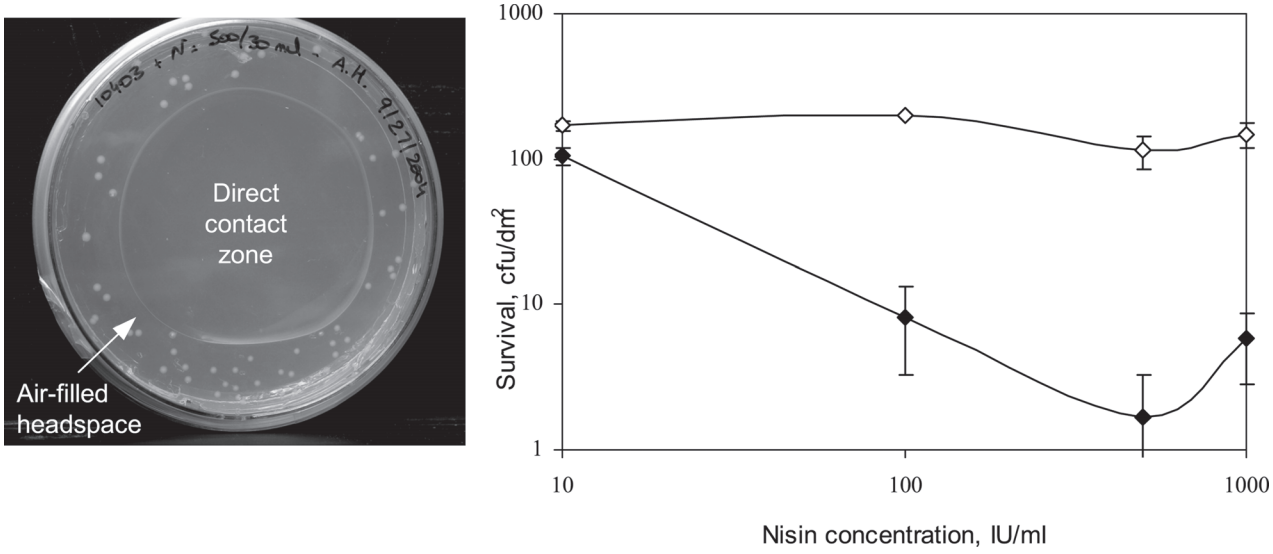

Figure 6. Colonies formed on TSA for the sample with 500 IU/ml nisin in the agar layer (left), and survival of* L. monocytogenes *(right): ◇—under the air filled headspace, ●—under the agar layer with various concentrations of nisin.

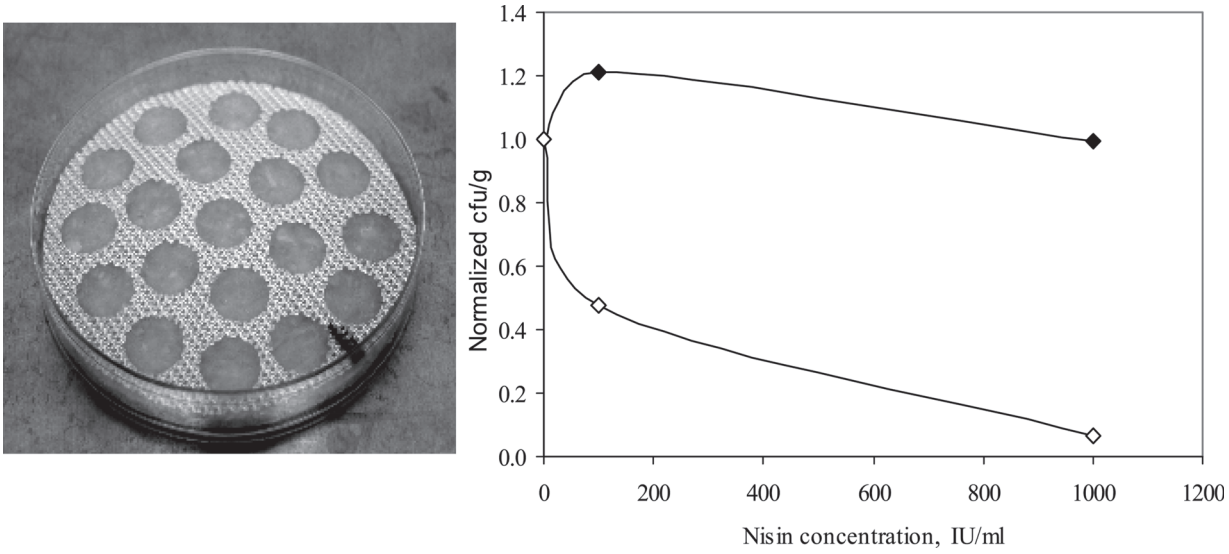

Figure 7. Nylon spacer used to model liquid headspace (left), and growth of* L. monocytogenes *on sliced turkey (right) at various concentrations of nisin: ◆—with the liquid-filled headspace, ◇—under the agar layer with no headspace.

bacterial growth than the samples with no headspace. The growth in the 1000 IU/ml sample with headspace was higher by almost one fold compared to the sample with no headspace.

These results show that headspace decreases the level of bacterial inhibition, probably because it limits the AMA transport from the packaging to the food surface. Therefore, the presence of liquid-filled headspace and its effect on the inhibition of bacterial growth should be taken into account when studying the efficacy of active packaging.

DISCUSSION

A model food/packaging system has been developed to investigate material independent AMA release efficacy. The agar matrix allows controllable and homogeneous release of the AMA; the release rate of the active compound can easily be quantified using the mathematical model developed. The system has been tested on TSA and model food product; the bacterial growth inhibition has been quantified by direct plate counting. Consistent inhibition levels have been observed with nisin concentrations tested, and good correlation was obtained with the standard agar diffusion test.

Depending on the environment and on the nisin load, some bacteria can develop resistance to the antimicrobial agent. The change in their sensitivity is due to changes in the fatty acid composition of the membrane of the resistant cells (Mazzotta and Montville, 1997). Numerous factors can influence the development of nisin-resistant bacteria: the dose of nisin, the method of its application, combination with other treatments, etc. Development of the mutants explains observed overgrowth of *L. monocytogenes* at high AMA loads (see Figure 5 and Figure 6).

One can recognize two characteristic timescales for antimicrobial delivery through the packaging headspace δ : diffusion migration time

$$t_{diff} \approx \frac{\delta^2}{D} \tag{6}$$

where D is AMA diffusivity, and characteristic time for bacteria reproduction:

$$t_b \approx \frac{1}{\mu} \tag{7}$$

where μ is the growth rate of bacteria.

Therefore, to prevent bacterial growth, AMA should be delivered through the packaging headspace faster than bacterial population growth. In other words:

$$t_{diff} < t_b \tag{8}$$

One can estimate a critical thickness for the headspace as following:

$$\delta_{cr} = \sqrt{\frac{D}{\mu}}$$

If $\delta < \delta_{cr}$ headspace has no effect on the effectiveness of antimicrobial control release from the packaging material and packaged food can be considered in direct

contact with packaging. Increase of headspace ($\delta > \delta_{cr}$) results in delayed delivery of AMA and decreased efficacy of inhibition. This means that to inhibit bacterial growth one will need to deliver increased amount of antimicrobial agent, which results in higher processing costs and lower food quality.

CONCLUSION

We have introduced a simple model food/packaging system to study the efficacy of controlled AMA release on bacterial growth inhibition. The system designed permits considering the effect of headspace, either liquid- or gas-filled. Both types of headspace showed significant decrease in the bacterial growth inhibition due to limited AMA transport from the packaging layer to the model food. The model food/packaging system has been validated on a ready-to-eat meat product (sliced turkey), and reasonable bacteria inhibition levels were achieved. The influence of headspace, food matrix and packaging design can be tested with this system, which provides important information for the development of active packaging and characterization of antimicrobial release.

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Application of Fluid and Statistical Modeling to Establish the Leak Size Critical to Package Sterility

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ABSTRACT: Onset of liquid flow through a defect as a result of imposed pressures or vacuum is shown to be linked to the sterility loss of a package. Five-hundred sixty-seven test cells with microtubes of 0, 2, 5, 7, 10, 20 or 50 μm, were manufactured to simulate packages with defects. They were biochallenged via an aerosol concentration of 10^6 cells/cm^3 of *Pseudomonas fragi* L-1052, under conditions of imposed pressure or vacuum of 20.7, 13.8, 6.9, 0, –6.9, –13.8, –20.7 kPa, respectively and temperatures of 4°, 25° and 37°C. A statistically significant relationship between loss of sterility due to microbial ingress in test cells and the initiation of liquid flow were found ($p < 0.05$). Microbial ingress was not found in test cells with microtube internal diameters (IDs) of 2 μm under any conditions. Leak sizes critical to sterility maintenance were based on the relationship between liquid surface tension and imposed pressure. Threshold leak sizes where the onset of liquid flow was initiated, and critical leak sizes at which loss of sterility occurred, were not significantly different ($p > 0.05$).

INTRODUCTION

STERILITY maintenance assurance continues to be a prominent concern for producers of aseptically packaged products. Such producers have aggressively embraced new technologies to manufacture flexible and semi-rigid packaging. Although many technologies have been developed to maintain package sterility, a problem that remains unresolved is the identification of the *critical leak size*. The critical leak size is that at which container sterility is jeopardized [8].

Presently, differences can be found in the scientific literature regarding the critical leak size. Howard and Duberstein [14] found that under specific conditions certain types of water borne bacteria penetrated 0.2 μm membrane filters and therefore speculated that the minimum hole size critical to sterility maintenance and integrity of the package, or the critical leak size, is between 0.2 μm and 0.4 μm. This range was selected based on the size of membrane filters used routinely for aseptic packaging and clean room applications with little significant microbial contamination. Lampi [20] demonstrated bacterial penetration via holes of less than 10 μm was unlikely. Lake et al. [19], during an extensive 4-year study, found leaks must be considerably larger than 1 μm for bacterial penetration to occur. Gilchrist et al. [11] showed bacterial contamination of cans from cooling water requires pinholes larger than 5 μm. McEldowney and Fletcher [23, 24] found that holes of 1 μm permitted microbial entry under certain conditions. Chen et al. [6] observed that 5 μm pin holes allowed microbial aerosol penetration. Board [4] found the pores of eggs (7 μm and 10 μm IDs) would permit microbial ingress when washed in liquids warmer than the egg or when stored in conditions of high relative humidity. Jarrosson [16] found that a 20 μm diameter hole with a 5 mm channel length permitted microbiological contamination in Meal Ready to Eat (MRE) pouches.

Much of the discrepancy over the threshold leak size in the aforementioned studies rest in the inability to manufacture and maintain the integrity of the leak size during the process of experimentation. The problem is

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further magnified for small hole diameters such as 10–20 µm internal diameters (ID) [12, 16].

Differences can also be found with regards to the leak sizes which are readily detectable using current on-line technology versus the speculated value for the critical leak size. It has been suggested that package inspection systems are available which can provide sufficient safety assurance and detection for microleaks with IDs of 10 µm for pinholes and 50 m for channel leaks [33]. However, the critical leak size is believed to be 10 µm for channel leaks [3]. The emphasis of research found in the scientific literature is sharply focused on package leak detection [2, 11, 22, 27, 29, 31].

A paradigm shift in package integrity research is currently underway. The emphasis of the most recent research suggest that physical factors, such as the development of a leaker, are responsible for the loss of package sterility [2, 17, 23, 24, 25]. An equation was established to quantify the forces required to initiate flow of a liquid of a given surface tension, through a defect with a known diameter, to produce a leaker [18]. The defect size at which the onset of liquid flow is initiated is called the threshold leak size [18].

After liquid flow initiation, a liquid pathway through the defect linking the interior of a package to the exterior may be present [18]. Liquid food product on the outside of a package as a result of passage through a defect from the package interior has been long suspected of facilitating post-process contamination [2, 19, 25, 29]. The incidence of post-process contamination is well documented, however, the mechanism by which post-process contamination occurs remains unquantified [19, 27, 30, 31].

In this study, a model for the initiation of liquid flow and the threshold leak size will be used in an effort to establish a relationship between the threshold leak size, the critical leak size, and loss of package sterility [18]. Variables of temperature, imposed pressure and vacuum were examined to determine their relationship to the leak size critical to the sterility of a package.

MATERIALS AND METHODS

Microtubes

Nickel microtubes were supplied by the Phillips Laboratory, Fundamental Technology Division, Carbon Research Laboratory, Edwards Air Force Base, CA through a Cooperative Research and Development Agreement [13]. Sixty-three (nine of each size) microtubes with IDs of 0, 2, 5, 7, 10, 20 and 50 µm and 7 mm in length were used as the manufactured defects (Figure 1) . Solid microtubes were used as a control.

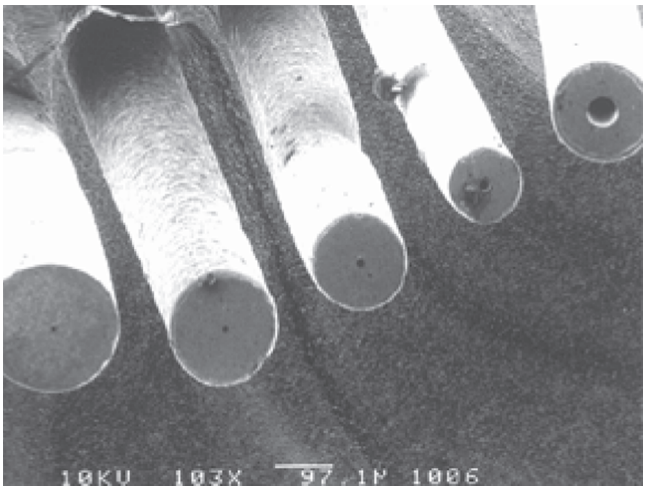

Figure 1. *Electron micrograph showing end views of nickel microtubes with Ids of 2 µm, 5 µm, 7 µm, 10 µm, and 50 µm. Microtubes are 7 mm in length.*

Exposure Chamber

The exposure chamber was constructed of Lexan® in dimensions of 35-cm (L) × 25-cm (W) × 25-cm (H). The internal area of the exposure chamber is 21,875-cm^3, and is divided into two sections: 1) top; utility section, 2) bottom, the exposure section (Figure 4). The utility section (dimensions: 35-cm [L] × 25-cm [W] × 18-cm [H]; total area = 15,750-cm^3) housed the vacuum, water input and recovery manifolds, all related tubing, vacuum and compressor tubing, as well as the test cells (Figures 2 and 3). The neck of each test cell passed through one of seven 2.85-cm holes in the partition. The top and end panels of the exposure chamber had stainless steel handles and were removable. The entry ports were created using two brass male and female threaded fittings with rubber O-rings.

The exposure section (dimensions: 35 cm [L] × 25 cm [W] × 7 cm [H]; total area = 6125 cm^3) has entry ports positioned in the center of opposing panels of the exposure chamber for bioaerosol delivery. Nebulizer kits (Baxter model 2D0807, Toronto, Ontario, Canada) with a mass median aerodynamic diameter of 2.68 µm, a geometric standard of 1.85 µm, and a mass of aerosol per minute of 1.1 µm and 4.7 µm were used. The maximum air flow (ml/h) at 10 Lpm was 21.9-ml/h. Four 6.9-m^3 size E cylinders, each equipped with CGA 346 air (0–15 Lpm) flow meters were used for the air sup-

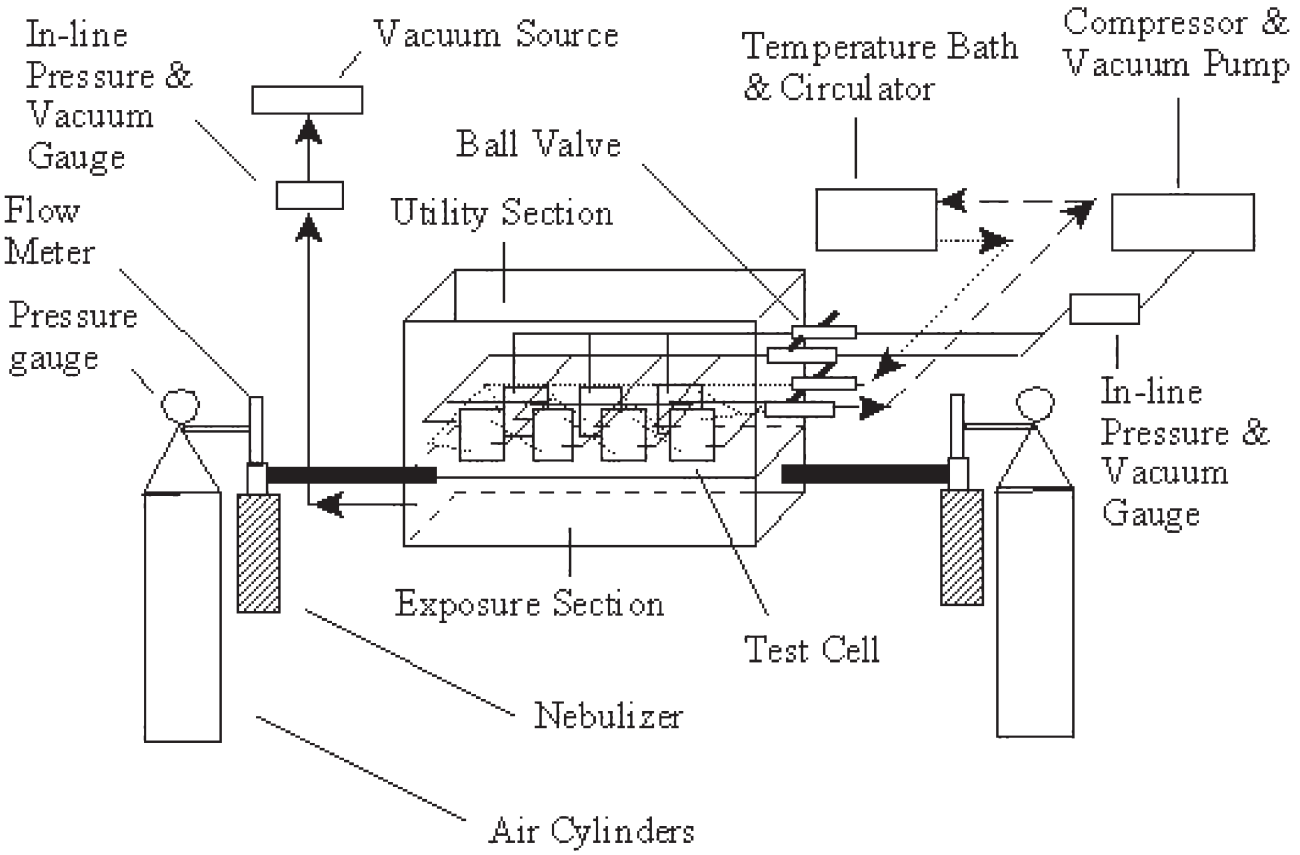

Figure 2. *Diagram of equipment set-up for bioaerosol changes of test cells in the exposure chamber.*

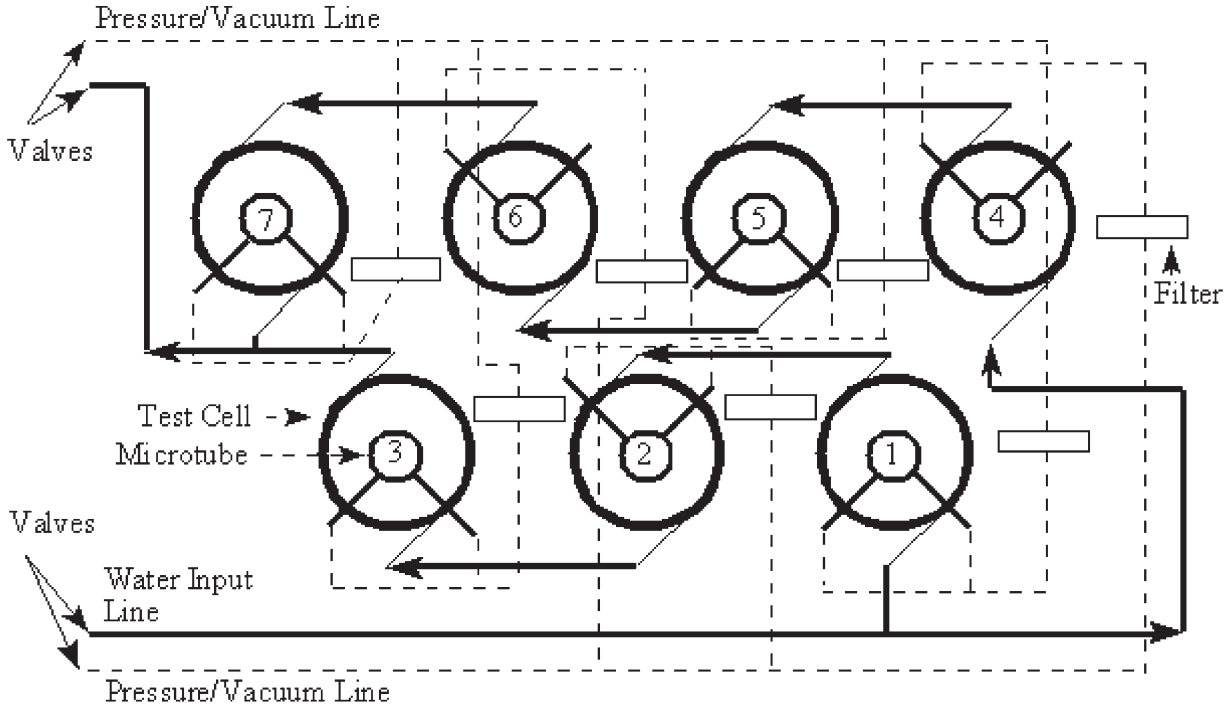

Figure 3. *Schematic of exposure chamber utility section showing test cell positions 1-7, water, imposed positive pressure/vacuum input, exit manifolds, valves, and in-line filters.*

ply. Twenty-one 37-mm, 0.33 μm inline bacterial air vents (product no. 4210, Gelman Sciences, Ann Arbor, MI) were used to filter air flowing into the sterile test cells. The bacterial vents were also used on the pressure equalization ports for the utility and exposure sections of the exposure chamber. Pressure inside the exposure chamber was equilibrated and maintained at room pressure using 0.41 kPa of vacuum.

Test Cells

Glass test cells were developed for the purpose of simulating imposed pressure within a package while maintaining sterility. The glass test cell dimensions are 8-cm [H] × 5-cm [D]. Each test cell consists of a 45-mm [H] × 15-mm [D] glass vial (3-ml capacity) encased in a 85-ml glass water jacket. The vial and the jacket have one entry and one exit port each. The vial has a glass lug for a septa closure [18].

Test Organism

Pseudomonas fragi L-1052, selected from the collection of G. H. Lacy, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA was used as an indicator of sterility. Pseudomonades are aerobic, gram-negative, motile, nonsporeforming, catalase-positive rods with polar flagella, ranging from 0.5–1.0 μm in diameter and 1.5–5.0 μm in length. Optimal temperature and pH ranges are 25°–30°C and 6.6–8.5, respectively. *Pseudomonas fragi* L-1052 resistant to kanamycin (30 μg/ml) and tetracycline (10 μg/ml).

Preparation of Test Cells

Microtubes were positioned inside a 27-gauge syringe needle. The needle was used to puncture and penetrate through the center of a silicone septum. The syringe needle was removed, leaving the microtube in place. The internal diameter of each microtube was measured to obtain the cross-sectional area using a light microscope (Olympus Model BH-2, Lake Success, NY) equipped with video callipers.

Septums containing microtubes were positioned on top of the test cell finish. Glass lugs of the test cell were wrapped with teflon tape, overlapping the top outside circular edge of the septum. Septa caps were placed over the septums and tightened. DAP™ silicone sealer (Dow Corning, Dayton, OH) was used to seal the septa surface around the microtube and the septum-septa cap contact area. Seal integrity of test cells were confirmed using the safranin red dye test, bubble leak test, and vacuum test [18].

Preparation of Exposure Chambers

Seven test cells, each with a microtube of a different internal diameter, were randomly assigned one of seven positions within the exposure chamber. Each test cell was filled with 3 ml of tryptic soy broth (Difco Laboratories, Detroit, MI) inverted to a septa cap down position, and secured on top of a rubber gasket. All rubber gaskets received a thin coat of high vacuum grease (Dow Corning Corp., Midland, MI) on both the top and bottom surfaces. Test cells were secured to the partition by bolt-down aluminum brackets. General purpose 6.4 mm ID rubber tubing (Fisher Scientific, Atlanta, GA) with a wall thickness of 2.4 cm, were connected to the entry and exports of the vial section of each test cell. Tubes were equipped with an in-line bacterial air vent (# 4210, Gelman Sciences, Ann Arbor, MI) positioned inside the holding section to prevent contamination during post autoclave cooling. Each tube was also connected to a brass ball valve, on the exterior of the chamber. The exposure chamber was positioned so that septa caps faced up to eliminate contact of the microtubes with the liquid tryptic soy broth (TSB; Difco, Detroit, MI) within each of the test cells during autoclaving.

Exposure chambers were autoclaved separately, at 121°C for 55 min. Valves on each exposure chamber were opened prior to autoclaving to facilitate sterilization and to dampen the generation of pressure differentials between the test cells, the exposure chamber and the autoclave.

Prior to the introduction of the bioaerosol into the exposure section, each exposure chamber was inverted so that the septa caps faced downward. The inverted position of the test cells facilitated continuous contact of the liquid growth medium with the microtubes.

Preparation of Bioaerosol

Inocula of 10^9 CFU/ml *Pseudomonas fragi* L-1052 motile organisms were prepared according to Keller et al. (17). The optical density of the TSB with the test organism growth was measured and compared to a standard. Two cartridges, each filled with a source concentration of 10^9 cells/mL of *Pseudomonas fragi* L-1052 in

200-ml of TSB were centrifuged at 16, 270 × g for 10 min (RC-5B, Sorvall Instruments, Newtown, CT). The tryptic soy broth was aseptically decanted and replaced with 200 ml of sterile phosphate buffer adjusted to a pH of 6.8 to 7.0. The optical density of the decanted TSB was measured and compared to a standard. Cartridges were oscillated for one minute to resuspend the microorganisms into the solution. The final challenge suspensions were aseptically transferred to the reservoirs of the nebulizer kits.

Aerosol Biochallenge

Nebulizers were secured to the external ports centered on each of the two small end panels of the exposure chamber via a sterile 2.54 cm ID × 3.2 cm OD clear PVC tubing (Nalgene, Fisher Scientific, Atlanta, GA) with a wall thickness of 3.2-cm × 45 cm in length. The exposure period was divided into a 30-min come-up period, and a 5-min static period.

The 30-min come-up period is the time required to achieve the final desired bioaerosol concentration of 10^6 CFU/cm^3 within the exposure section. A total of 6 ml of the source concentration of approximately 10^9 CFU/ml were introduced via aerosol into the 6, 125 cm^3 exposure section, for a final airborne concentration of approximately 10^6 CFU/cm^3. A vacuum of 0.42 kPa was used to maintain pressure equilibrium between the exposure chamber and ambient conditions.

Imposed Pressure and Vacuum

To measure the influence of imposed pressures or vacuums on the critical leak size, positive pressures or vacuums were imposed on the internal vial of the test cell at the onset of the come-up period. Internal positive pressures of 6.9, 13.8, or 20.7 kPa, or a vacuum of –6.9, –13.8, or –20.7 kPa were imposed on the test cells at 25°C. Positive pressures and vacuums were imposed via a compressor/vacuum pump (model ROA-P131-AA, Gast, Benton Harbor, MI). General purpose rubber tubing (Fisher Scientific, Atlanta, GA) with 37 mm, 0.33 µm inline bacterial air vents (product no. 4210, Gelman Sciences, Ann Arbor, MI) were used to communicate the imposed pressure from the compressor to the test cell. A 500 ml vessel with brass entry and exit barbed ports were used to stabilize the air flow from the compressor to produce pressure fluxuations of ≤0.06 kPa. An in line pressure gauge (Model HHP701-2, Omega Engineering, Inc., Stamford, CT) with a detection range of 137.9 kPa of vacuum or positive pressure, and a resolution and accuracy of 0.05% and ± 0.15% FS, respectively, was used to measure the applied imposed pressure.

After completion of the come-up period, the bioaerosol and vacuum were discontinued, thus initiating the static period. Each chamber remained in the static period for 5 minutes at a temperature of 25°C. The static period provided time for the aerosol to initiate fall out as a result of natural sedimentation [9, 21, 28, 30]. Aerosol residual was removed via an exposure section vent port with an in-line bacterial vent under 20.7 kPa of vacuum.

Exposure chambers were incubated for 72 h at 25°C. Following the incubation period, test cells were removed from the utility section. Turbidity of TSB within a test cell indicated a positive for loss of sterility. To confirm that loss of sterility was due to ingress of the test organism, liquid samples were aseptically transferred and plated from test cells showing turbidity and plated on TSA with kanamycin and tetracycline, 30 µg/mL and 10 µg/mL, respectively.

Imposed Temperature

To determine the effect of temperature on the critical leak size independently of pressure, vacuums or positive pressures were not imposed on test cells challenged under temperature conditions. Test cells were challenged under temperature conditions of 4°C, 25°C, or 37°C at 0.0 kPa. Temperature control was maintained using a 1:1 v/v, solution of water and ethylene-glycol using a variable temperature bath and circulator (Masterline™ Model # 2095, Forma Scientific, Marietta, OH). Target temperatures were maintained for one hour prior to biochallenge initiation.

Relative Humidity

The relative humidity created by nebulizers within the exposure section of the chamber during a simulated bioaerosol challenge (without test organism) was measured. Relative humidity was measured by eight thermocouples: four-wet-bulb, each with moist, 100% cotton covers, and four-dry-bulb. Paired wet-bulb and dry-bulb thermocouples were secured in the centers and on a side wall of both the exposure and holding sections. Distilled water was introduced into the exposure

section of the chamber at a combined flow rate of 20 Lpm (i.e., 2 nebulizers @ 10 Lpm each) for 30 min.

Confirmation of Airborne Microorganism Concentration

To verify that the concentration delivered to the microtubes attached to the test cells was a 10^6 cells/cm^3 concentration, filter papers (product no. 63077, Gelman Sciences, Ann Arbor, MI), were positioned within the exposure chamber to catch settling airborne microorganism from the bioaerosol. Plate counts from the filter papers were measured against plate counts obtained as a result of aerosol trapping during chamber evacuation.

Filter papers were attached via sterile water contact to test cell positions, the bottom center, and to the geometric center of a long side panel in the exposure section of the chamber. A filter was attached on the partition within the utility section of the chamber as a control. The chamber was autoclaved at 121°C for 55 min and allowed to cool to 25°C.

The exposure section of the chamber was subjected to a bioaerosol with a 10^9 CFU/mL source concentration of *Pseudomonas fragi* L-1052 for 30 min. Bioaerosol residual was evacuated under 20.7 kPa of vacuum into a 1 L flask filled with sterile water containing peptone. The contents of the flask were serially diluted and plated.

Filters were aseptically transferred to bottles containing 100 ml sterile water with peptone. Each blank was placed on a shaker, model G-2 (Gyrotory™, New Brunswick Scientific Company, Inc., Edison, NJ), for 1 min. The contents of seven blanks were serially diluted, plated and incubated at 28°C for 24 hours.

Temperature Verification of Test Cells

Temperature verifications were performed to determine the time required to achieve temperatures of 4°C and 37°C from a starting temperature of 25°C. Thermocouples were inserted into the center of each septum of the seven test cells with ends poised 1.5-cm from the septum surface facing inward. Each test cell was filled with 3 ml of TSB and positioned inside the exposure chamber.

Experimental Design

A randomized complete block design was employed. The purpose of the block design was to independently measure the influence of positive pressure, vacuum, and temperature on the threshold defect size critical to the sterility maintenance of a package.

Seven test cells with one microtube of each available size were represented within each exposure chamber. To reduce the potential for a position effect, the order of test cells were randomized. Each exposure chamber was replicated. Nine randomized replicates were challenged via bioaerosol per imposed pressure, vacuum or temperature condition (i.e., 9 conditions; +20.7, +13.8, +6.9, 0, –6.9, –13.8, –20.7 kPa at 25°C, and 0 kPa at 4°C and 37°C).

Two models were employed in this study; the equation for the initiation of liquid flow (M1), and a statistical model (M2). M1 was used for the calculation of threshold imposed pressures required to initiate liquid flow per microtube ID size:

$$P_o > P_{ATM} + [(4\ \mu/D_H - \Delta gL) \times 0.395] \qquad (2)$$

where the imposed pressure (P_o) must be greater than the surface tension (μ) for a given hydraulic diameter (D_H) with a given static head (ΔgL) [18]. When threshold imposed pressures are met or exceeded, a liquid pathway through the microtube is established, indicating the threshold leak size [18].

M2 was designed for a fixed temperature/pressure combination in a logistic regression analysis:

$$p = \begin{cases} (1+\exp(\beta_0 + \beta_1(Size - e^T)))^{-1} & \text{if } Size - e^T \geq 0 \\ 0 \text{ otherwise} \end{cases} \qquad (3)$$

where is the portion of test cells that realized ingress, 0 is the intercept, 1 is the slope, and e^T is the threshold value [5].

It is also of interest to approximate the relationship between the threshold defect size and pressures. The following model in a binary regression analysis was used [5]:

With this parameterization, the threshold defect size is estimated as the following function of pressure for a

$$p = \begin{cases} 1 - \exp^{-\exp(\beta_n + \beta_1(Size - \exp^{(c_0 + c_1 Pressure + c_2 Pressure^2)}))} \text{ if } Size - \exp^{(c_0 + c_1 Pressure + c_2 Pressure^2)} \geq 0 \\ 0 \text{ otherwise} \end{cases} \qquad (4)$$

given temperature [5]:

$$\exp^{(\hat{c}_0+\hat{c}_1\ Pressure\ +\hat{c}_2\ Pressure^2)} \quad (5)$$

The method of maximum likelihood was used to estimate the parameters in each of the model equations listed above. This model was designed to relate the proportion of test cells identified as positives for microbial ingress to the microtube ID. This allowed a critical leak size per a set of conditions to be established.

Statistical Analysis

A non-linear regression was used for data analysis to determine significance between threshold imposed pressures per microtube ID size by M1, predicted values for the critical leak size predicted by M2 versus observed critical leak sizes. Analyses were carried out using JMP® (SAS Institute, Cary, North Carolina).

RESULTS AND DISCUSSION

Test Cells

Test cells with solid microtubes showed no indications of microbial contamination. This confirmed that test cells positive for microbial contamination resulted due to ingress through holes in microtubes.

Relative Humidity

A relative humidity of 98% ± 1% was achieved within three minutes of iniation of nebulizers. The target relative humidity was 55%. Maintenance of high relative humidities is important for challenge tests that employ bioaerosols. Relative humidities 32% result in erratic aerosol particle size, poor aerosol distribution, reduction of airborne microbial population and difficulties in experimental reproduction [7, 9, 10, 15, 28].

Confirmation of Airborne Microorganisms Concentration

Plate counts of *Pseudomonas fragi* L-1052 for filter papers extracted from the side panels and bottom center of the exposure section of the chamber were 5.3×10^5 CFU/ml to 1.5×10^6 CFU/ml, respectively, with an average of 8.7×10^5 CFU/ml. Plate counts of the bioaerosol evacuated form the exposure section of the chamber were 2.8×10^5 CFU/ml to 3.5×10^7 CFU/ml with an average 2.5×10^6 CFU/ml and $< 10^7$ CFU/ml. This confirmed that the target airborne microorganism concentration of 10^6 cells/cm^3 was achieved using a bioaerosol.

Temperature Verification of Test Cells

Temperature verification for test cells biochallenged at 4.4°C and 37.7°C were performed. For test cells with a target temperature of 4.4°C, an average temperature within test cells of 5.1°C ± 0.3°C was achieved in 126 min from a starting temperature of 23.8°C. For test cells with a target temperature of 37.7°C, an average temperature within the test cells of 36.8°C ± 1.6°C was achieved in 28 min from a starting temperature of 23.8°C.

Biochallenge via Aerosol Under Imposed Pressure and Vacuum

Threshold imposed pressures necessary for flow initiation of TSB with a surface tension of 44.09 mN/m through microtubes of 2, 5, 7, 10, 20 and 50 ?m were experimentally correlated to the onset of ingress under similar imposed pressures (Tables 1 and 2). For example, the imposed pressure required to initiate the flow of TSB through a 20 µm ID microtube was 3.45 kPa. Ingress was found in test cells with a microtube ID of 20 µm under imposed pressures > 3.45 kPa.

An imposed pressure of –13.8 kPa resulted in 19 of 54 tests cells positive for microbial ingress contamination. Ambient pressure conditions (0.0 kPa) resulted in two positives for contamination of 54 test cells (Table 1). Microbial ingress occurred in test cells where the pressure or vacuum required for the initiation of flow of TSB or distilled water, were met or exceeded. Water was important in that it was employed to transport the test organism to the test cells in the exposure chamber. Therefore, the surface tension of water was an important consideration for the process of ingress into test cells under imposed vacuums. To illustrate, the surface tension of water is 64.67 mN/m and requires an imposed vacuum of –11 kPa to initiate flow through a microtube with an ID of 10 µm. Microbial ingress was found in test cells where the imposed vacuum exceeded –11 kPa (Table 1).

Imposed pressures of –13.9 kPa and -20.7 kPa resulted in four positive test cells for microbial ingress of 9, and 1 positive of 9, respectively. The number of positives did not increase with increased imposed vac-

Table 1. Microbial ingress into test cells as a result of bioaerosol exposure of 30 minutes come-up period, and a 5 minute static period with imposed pressures with in the test cells of –20.7, –13.8, –6.9, 0, –6.9, 13.8, and 20.7 kPa at 25°C. Sixty-three test cells of each microtube diameter were bio-challanged.

	Imposed Pressure (kPa)							
Microtube ID Size (μm)	**–20.7**	**–13.8**	**–6.9**	**0**	**6.9**	**13.8**	**20.7**	**Total Positives**
50	4/9	8/9	1/9	2/9	3/9	1/9	3/9	22/63
20	6/9	4/9	0/9	0/9	1/9	6/9	6/9	23/63
10	1/9	4/9	0/9	0/9	0/9	3/9	3/9	11/63
7	0/9	1/9	0/9	0/9	0/9	3/9	1/9	5/63
5	1/9	2/9	0/9	0/9	0/9	3/9	1/9	7/63
2	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/63
Total Positives	**12/54**	**19/54**	**1/54**	**2/54**	**4/54**	**16/54**	**14/54**	**68/378**

uum. These findings agreed with that of McEldowney and Fletcher [25] in that the number of positives for microbial ingress found in their study were not proportional to the magnitude of imposed vacuum.

The incidence of ingress was highest in test cells with microtubes of 20 mm ID, where 23 of 63 test cells resulted positive for microbial ingress contamination. The lowest incidence of microbial ingress was 0 positives of 63, found for test cells with a microtube of 2 μm ID (Table 1). The hydrophilic nature of the nickel microtubes may have contributed to a low critical leak size. Larger critical leak sizes may have resulted if the microtubes were constructed of hydrophobic materials, such as polyethylene or polypropylene. Surface oxidation of nickel microtubes may facilitate fluid movement by creating a hydrophilic surface, potentially reducing the imposed pressure required for the initiation of flow.

For microtubes with IDs of ≤ 20 μm, imposed pressures of 6.9, 13.8, and 20.7 kPa produced a total of 27 positive test cells for microbial ingress versus 19 test cells for imposed pressures of –6.9, –13.8, and –20.7 kPa. Ingress was found for test cells with a microtube ID of 20 μm under an imposed pressure of 6.9 kPa. However, ingress was not found in test cells with a microtube ID of 20 μm under an imposed pressure of –6.9 kPa. The difference between values for positives between pressure conditions of 6.9 kPa and –6.9 kPa were a function of differences between liquid surface tensions of TSB and water. Surface tension values for TSB inside the test cells were lower than those of distilled water forming the aerosol used to transport the test organism; 44.09 mN/m compared to 64.67 mN/m [18]. Therefore, TSB required less imposed pressure than water to initiate flow through microtube IDs used in this study.

Imposed pressure conditions of –13.8 and –20.7 kPa produced 12 positives for microbial ingress for test cells with microtubes IDs of 50 μm versus four positives for identical test cells under imposed pressures of 13.8 and 20.7 kPa. Under most pressure conditions, fewer positives were found for test cells with 50 μm ID microtubes than for test cells with 20 μm IDs (Table 1). An explanation rests in the dynamics of droplet formation [33]. For microtubes with 50 μm IDs, positive pressures greater than 6.9 kPa incite a rapid increase in droplet size [18]. When the droplet reached a sufficient size (a diameter of approximately 900 μm), detachment from the microtube occurred. As a result, microorganisms transported via bioaerosol and contacting the droplet were carried away from the microtube by the detached droplet, thwarting ingress into the test cell.

The absence of microbial ingress in test cells with microtube IDs of 2 μm can be explained via the relationship between the fluid surface tension and imposed pressure in relation to the hole size. The imposed pressure required to initiate the flow of TSB through a microtube ID of 2 μm is 39.29 kPa (Table 2). A maximum imposed pressure and vacuum of 20.7 kPa were used in this study. Therefore, microbial ingress was not found in test cells with a microtube ID of 2 μm because the maximum imposed pressure used, 20.7 kPa, did not exceed 39.29 kPa which is required to initiate the flow of TSB.

The threshold imposed pressure or vacuum required to initiate flow of TSB and distilled water produced by M1, coincided with those associated with critical leak values (Table 2). Packages with a partial vacuum or that maintain constant pressure differences between the inside and the outside have been found to be at greater risks for contamination than those at atmospheric pressure [23, 29]. Banks and Stringer [2] found that bacterial transfer through a 5 μm diameter channel leak was higher when a vacuum was applied. The findings of this

Table 2. M1 predicted values for liquid flow and the imposed pressures at which microbial ingress was found for tryptic soy broth with a surface tension of 44.09 mN/m, through microtubes of 50, 20, 10, 7, 5, or 2 μm at 25°C.

	Imposed Pressures (P_o)	
Microtube ID Size (μm)	M1(kPa)	Ingress for Imposed P_o (kPa)
50	1.47	6.9/–6.9
20	3.9	6.8/–13.7
10	8.22	13.7/–13.7
7	11.66	13.7/–13.7
5	15.43	13.7/–13.7
2	31.80	No Ingress

study agree with that of McEldowney and Fletcher [23] in that packages with large vacuums may be at no greater risks than those with low internal vacuums. Data in this study suggest that packages under positive pressures may face greater risk than packages under vacuum. Such positive pressures may occur during distribution [18].

Effect of Temperature

The number of test cells with microtube IDs of 20 μm positive for contamination under imposed temperatures of 4.4°C and 37.7°C were 1 of 9 and 2 of 9, respectively (Table 3). The critical leak size for test cells challenged with a bioaerosol at a temperature of 25°C was 50 μm (2 of 9). Lower critical leak sizes resulted for temperatures of 4.4°C and 37.7°C due to differences between the temperatures of the exposure chamber and the test cell. Temperatures within the exposure section of the chamber decreased as relative humidity increased as a result of the bioaerosol presence. From a starting temperature of 25°C, the temperature within the exposure section was 20°C ± 1.5°C after 5 min of bioaerosol initiation. Since the temperature of the test cell (4.4°C) was lower than that of the atmosphere in the exposure section of the chamber (20°C), airborne particles exhibited thermophoresis by moving from a high temperature zone within the chamber to a lower temperature zone within the test cell [31]. For test cells biochallenged at a temperature of 37.7°C, the surface tension of the TSB within the test cells accounted for the increased number of positives for ingress compared to test cells biochallenged at a temperature condition of 25°C. An increase in the temperature of TSB above the start temperature of 25°C produced a decrease in surface tension, and allowed the initiation of liquid flow under ambient pressure conditions [26].

Critical Leak Size Comparison of M1, M2, and Observed Values

The critical leak size is the smallest microtube ID where microbial ingress was found per conditions of imposed pressures. Values for the M1, M2 and the observed values were significantly different from each other ($p < 0.05$). The logistic regression model (M2) predicted values for the critical leak size based on the observed data and did not consider physical properties of the liquid food product, such as surface tension. The liquid flow model (M1) predicted the threshold leak size based on the imposed pressure and surface tension of the liquid product. Analyses employing ANOVA indicate that the threshold leak sizes produced by M1, and the observed critical leak sizes were not significantly different ($p > 0.05$). Differences between the observed critical leak size and values produced by M1 are due to surface tension and the microtube sizes. Such was the case for the observed and predicted critical leak sizes under an imposed pressure of 20.7 kPa. The observed critical leak size under an imposed pressure of 20.7 kPa was 5 μm, compared to the M1 predicted of 3.9 μm. However, no microtube sizes between 2 μm and 5 μm were tested. An imposed pressure of 20.7 kPa was sufficient to initiate flow of TSB through a microtube with an ID size of 5 μm, but not sufficient to initiate flow through a microtube with a 2 μm ID. Therefore, the defect size critical to the sterility of a package can be calculated if the liquid surface tension and the internal pressures the package will encounter during distribution are known.

Gnanasekharan and Floros [12] suggested that factors such as length to diameter ratio of the leak, the internal geometry of the leak (straight/tortuous or smooth/rough) and the pressure differential across the

Table 3. Comparison of three temperatures (4°, 25°, and 37°C) effects, at 0 kPa, on the critical leak size.

	Temperature (°C)		
Microtube ID Size (μm)	4.0	25.0	37.0
50	3/9	2/9	2/9
20	1/9	0/9	2/9
10	0/9	0/9	0/9
7	0/9	0/9	0/9
5	0/9	0/9	0/9
2	0/9	0/9	0/9

leak interface, should be considered to determine the critical leak size for a package. However, Amini and Morrow [1] suggested that the diameter to length ratio of microleaks, for example, those of pin holes in the micron range found in thin foil, is of a sufficiently small magnitude so as not to require a correction factor. In this study, the microtubes used as leaker channels were 7 mm in length and straight. Previous research produced no evidence that supported significant effects on microbial ingress into a package via a defect as a function of channel length, although leak diameter itself was significant [16, 17].

In this study, the critical leak size was found to be a function of liquid avialability within, and through the microtube. Liquid TSB was present in and through the microtube when the imposed pressure required to initiate liquid flow was met or exceeded for each microtube ID size.

CONCLUSIONS

Many fluid foods have surface tension values similar to that of TSB [18]. This study, in conjunction with Keller et al. [18], produced data that establishes a relationship between the liquid surface tension of a food product, the imposed pressures the package will be expected to tolerate during distribution, and the threshold leak size. This, in part, explains the previously elusive nature of the critical leak size.

The critical leak size is a changing range, largely based on the surface tension, hole size and imposed positive pressure or vacuum, the package will be expected to tolerate during distribution. By averting such conditions sufficient to initiate flow of a fluid food product through a defect, such as alteration of its surface tension, reduction of defect size or avoidance of comparatively adequate imposed pressures, package sterility can be maintained.

The critical leak values produced in this study are potentially conservative in that they are smaller values than those that may be found using microtubes constructed of hydrophobic materials. Such values resulted as a function of the nickel used to construct the microtubes. Due to the possible presence of hydrophilic conditions as a result of surface oxidation of the nickel microtubes, less imposed pressure may have been required to initiate flow than for microtubes made of a material with hydrophobic characteristics. As a result, the value for the critical leak microtube ID may be smaller than those that would result using other materials found in the seal areas of aseptic packages, such as polypropylene.

The conservative critical leak values produced by this study are a result of the microtube placement. Prepared test cells are inverted prior to bioaerosol exposure, making the end of the microtubes the low points of the test cells. Therefore, the liquid within the test cells exerted the maximum static head pressure through the microtube, and facilitated the initiation of fluid flow. Defects found in the seal area on top of the package, for example, are not exposed to imposed pressures that result from static head.

In this study, a relationship between the imposed pressure required to initiate flow of a liquid through a defect and the loss of sterility has been established. For the first time, manufacturers of liquid food products can determine the leak size critical to the sterility of their product based on the liquid surface tension and imposed pressures required to initiate the flow of their product through the defect sizes commonly found in their packaging. As a result, the manufacturer may engage in a proactive approach to package sterility maintenance.

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An Overview of the State of Life Cycle Assessment and Its Application to Packaging

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ABSTRACT: Life cycle assessment (LCA) is a systematic approach to evaluating environmental burdens associated with a product, process, or activity covering its whole life cycle (i.e. from raw material acquisition to disposal) and is increasingly used by firms and government agencies to facilitate environmentally conscious manufacturing, pollution prevention, and "ecoefficient" or "green" design. This article provides an updated, comprehensive structured review of the state of LCA and its use by packaging oriented LCA practitioners, covering: the basics of LCA, limitations of current methods, enhancement approaches developed by the LCA community to address these limitations and specific applications in packaging, summarizing recent private and public examples with regard to LCA use and improvement initiatives.

INTRODUCTION

LIFE cycle assessment (LCA) is defined as a cradle-to-grave analysis that can be used to move towards the ideal of sustainable development [1]. Sustainability, as adopted by the World Commission on Environment and Development [2], describes the political goal for the future of mankind. Sustainable development implies an ideal balanced relationship between natural resources and human activity. LCA is intended to be an integral part in attaining this balance.

LCA is the result of the evolution of early "waste" and "energy analyses" performed by a few industries in the past. These waste-energy analyses were aimed at calculating the embodied energy and generation of solid wastes over different stages of the product life cycle. Later, due to their identical methodology, these were expanded to encompass the computation of total life cycle release of pollutants.

LCA is one of a number of environmental impact evaluation techniques and as such, it is recognized that it may not be appropriate for all situations. In fact, this tool presents limitations that are intrinsically related to the definition of the scope and interpretation of the systems that are being assessed, the modeling of emissions, fate and final impacts of substances released into the environment, and problems due to the data-intensive nature of the assessment. As a result, LCA is an evolving tool.

The purpose of this paper is to provide an updated, structured review of LCA information for packaging-oriented LCA practitioners. To help present the information, this paper has several sections. The first section describes the basic nature and characteristics of the LCA methodology, followed by a review of the limitations of LCA and recent enhancement approaches developed by the LCA community to address some of the limitations. Next, the use of LCA specifically in packaging applications is reviewed. A birds-eye description of related private and governmental initiatives with regard to LCA in packaging is included. The final section discusses the likely future of LCA applications in packaging.

Definition of LCA

In the last fifteen years, several definitions of LCA have been offered with minor variations from each other. Developed in 1991, the definition by the Society of Environmental Toxicology and Chemistry (SETAC)[3], was among the first ones and describes LCA as:

> "… an objective process to evaluate the environmental burdens associated with a product, process or activity

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> by identifying and quantifying energy and material uses and releases to the environment, and to evaluate and implement opportunities to affect environmental improvements. The assessment includes the entire life cycle of the product, process or activity, encompassing extracting and processing materials; manufacturing, transportation and distribution; use, reuse, maintenance; recycling and final disposal".

Since 1997, harmonization steps regarding a common LCA understanding have been made with the appearance of the International Organization for Standardization (ISO) LCA-related standards [4–8] within the 14000 Series of Environmental Management System (EMS) standards, and their subsequent rapid global adoption. ISO defines LCA as:

> "a compilation and evaluation of inputs and outputs and the potential environmental impacts of a product system throughout its life cycle".

In either of these definitions, the life cycle of the product system, or cradle-to-grave, starts with the gathering of raw materials from the earth to create the product and finishes when they are returned back to the earth. LCA then considers the cumulative environmental impacts that occur due to all stages of the product life cycle.

Worth noticing is the fact that by considering the whole life cycle of a product, LCA has the potential for avoiding shifting problems. However, understandable or not, the LCA definition does not require that all possible environmental impacts be accounted for (i.e. a study that looks only at greenhouse gas emissions on a cradle-to-grave basis can be called an LCA), allowing room for shifting burden.

Product Life Cycle

LCA is based on the product "life cycle". Figure 1 shows a simplified diagram of a typical product life cycle which would start with the raw material acquisition (e.g. petroleum extraction and refinery for petroleum-based plastic products). After raw material acquisition, the cycle would include the material manufacture stage. Here, raw materials would be processed into basic manufactured materials (e.g. manufacture of plastic resin pellets). These materials would then be moved to the actual product manufacture stage where they would be made into products (e.g. plastic pellets extruded and molded into milk jugs). Eventually, they would be used (e.g. by the milk distributor and consumer) and disposed. When disposed, they might go through waste management programs to be reused,

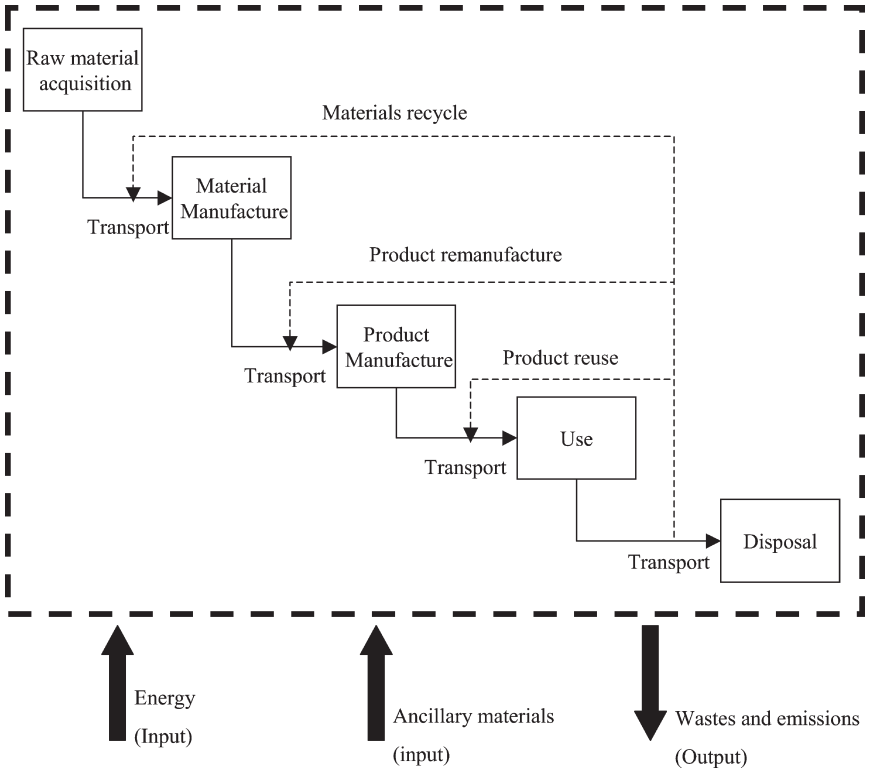

Figure 1. *Typical product life cycle.*

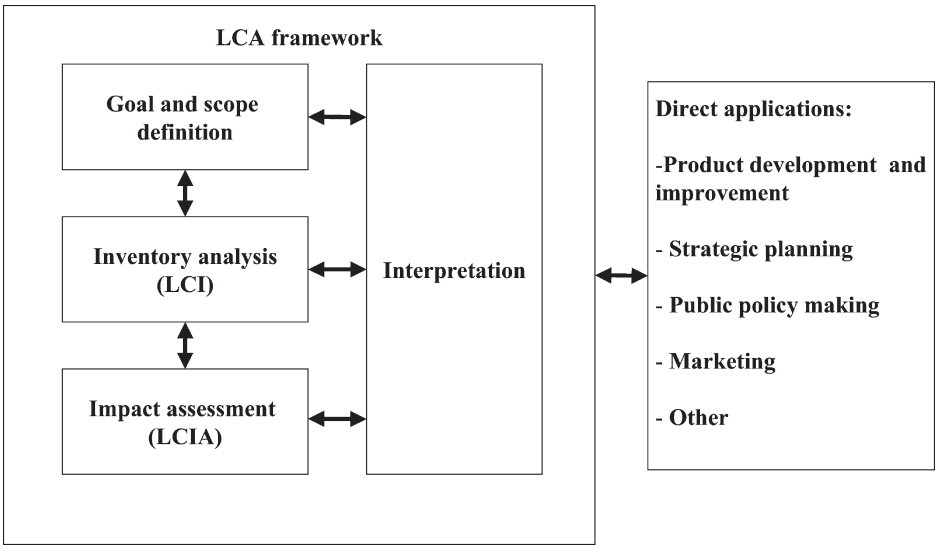

Figure 2. *The stages of LCA and possible applications [4].*

and/or recycled, and/or incinerated, and/or sent to landfills. As shown in the diagram, all stages are interlinked, and along with the transport required to move products and materials, require energy and ancillary materials and produce wastes and emissions.

LCA Stages

LCA is an evolving technique. Currently, ISO and SETAC guidelines divide the LCA into four stages [5] (see Figure 2):

Goal and scope definition: where the purpose of the study, its scope, functional unit and the procedure for quality assurance of the results are described. This step specifies the inputs and outputs selected for inventory and selects the functional unit, a common reference to which the inputs and outputs are related, associated with the function of the system under study.

Inventory analysis: which is the actual quantitative analysis of inputs of raw materials and fuels into a system and the outputs of solid, liquid and gaseous wastes from it. In the Life Cycle Inventory (LCI), data associated with the flows is collected using literature studies, interviews, measurements, theoretical calculations, data banks and qualified guesses. In theory, the application of allocation principles (i.e. partitioning the input or output flows of a unit process to the product system under study) and procedures should also be explained, and information required in recycle or reuse situations should also be presented. For transparency, the details of the methods for data collection and sources of the data should also be provided in this phase.

Impact Assessment: SETAC and ISO define environmental life cycle impact assessment (LCIA) as the stage whereby the inventory results are linked to the identifiable environmental problems. This stage is a technical, quantitative and/or qualitative process to characterize and assess the effects of the environmental emissions identified in inventory analysis.

There are a number of impact assessment methods [9], and related concepts and terminology are still being developed, but in general they include three basic steps: Step 1, identification of the potential environmental concerns (i.e. "impact categories") affected by the LCI component results; Step 2 classification, actual assignment of LCI results under the identified impact categories (one LCI component may affect more than one impact category); and Step 3 characterization, calculation of the contribution of the effect of LCI components to each identified environmental problem (i.e. category indicators).

There are two main approaches for estimating category indicators as outlined in Figure 3. A category indicator can be located at any place between the LCI results and the environmental "endpoint" [10].

One approach conforms to the so-called "mid-point" methods, which link the inventory results to environmental mid-point categories (e.g. ozone depletion or acidification or global warming potential). The term "midpoint" indicates that this point is located on the impact pathway at an intermediate location between the LCI results and the final environmental damage (i.e. endpoint). The alternate approach constitutes "end-point" or damage-oriented methods, which link

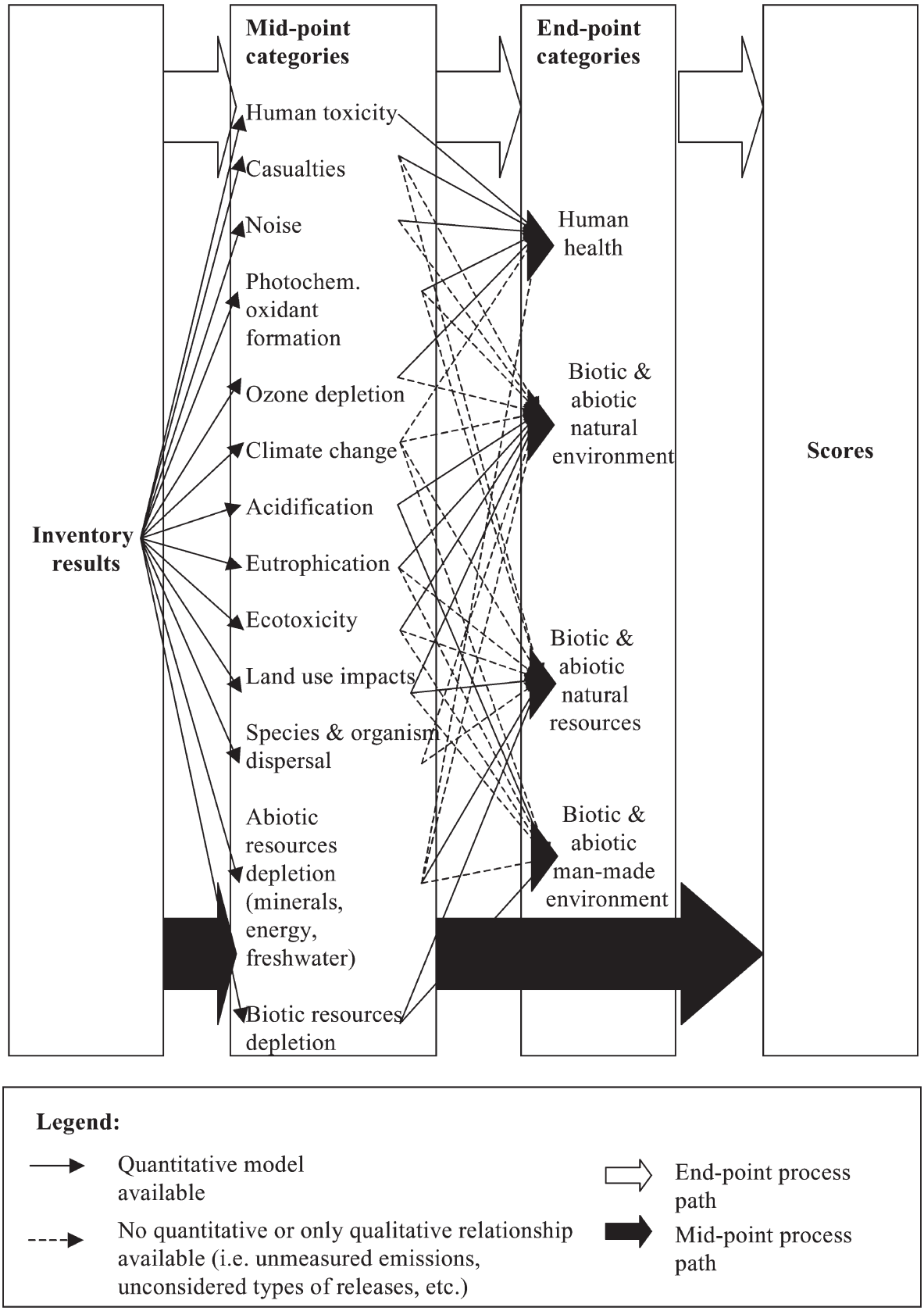

Figure 3. *General representation of main differences between mid-point and end-point methods for impact assessment (based on Bare et al [11] and Jolliet et al [10]).*

the inventory results all the way to damages (e.g. damage to human health or animal species endangerment). In doing so, an additional step may be used to allocate the previous "midpoint categories" into one or more "damage categories" [10].

After the characterization step, though not required by ISO standards, additional procedures are usually followed to better organize the results under some type of rating to facilitate decision-making. In particular, normalization, valuation methods and/or weighting procedures of the resulting impact outputs can be used in order to convert characterization results into "impact scores" with the intention of facilitating the decision-making process.

Interpretation: where the results from the LCI, alone or combined with those from the LCIA, are integrated to reach conclusions and recommendations. This stage may involve the iterative revision of the goals and scope of the LCA, and assessment of the quality of the data.

Limitations

There is almost unanimous opinion about the enormous potential of LCA. However, the current LCA technique, though gaining international acceptance, is far from perfect. One thought that probably can sum up the skepticism is by M. Densie [12]:

> "The outcome of the LCA is the result of the inputs. The inputs are the result of the preferences of those who are paying for the study".

Further, since there is not a single, harmonized and standardized approach of performing an LCA (e.g. due to differences in allocation rules, data collection procedures, etc.), any result can be challenged. This is critical when LCA is used as a product comparison tool to determine product preferability. In fact, Finnveden [13] argued that as long as no general framework is used in LCA, none of the studies can be used to show an overall environmental preference for any of the alternatives compared. The outlook is considered better when LCA is used as a tool for improving a system's environmental performance, since under the same approach on the same system, LCA could give useful information for strategic system improvement (e.g. for selecting the container size that appears to require the least life-cycle energy after analyzing the effect of using different container sizes to deliver a product to the consumer).

Shortcomings along the different LCA stages were classified by Huijbregts [14] who elaborated that LCA limitations arise mainly due to different kinds of uncertainties and variability.

In industrial systems, uncertainty (i.e. the lack of sureness about something) and variability (i.e. the inherent variation of measured values) are responsible for many of the problems in an LCA (see Table 1).

Uncertainty is important because when it is not evaluated, there is more risk that the impact predicted by the LCA will not match the actual environmental impact. Likewise, variability is important because it limits the precision of LCA results.

In the packaging field several of these limitations have been found to cause problems. For instance, Oki and Sasaki [16] describe how sometimes it is difficult to take into account the packaging function as a basis for comparison. The authors compare a gas-barrier multilayer container with a monolayer container and claim that the current state of LCA would exclude the gas barrier function from the assessment. That would make the monolayer material more desirable because it means less material consumption and less processing energy, and lower environmental burdens. Though it is true that the gas-barrier material involves more energy and costs more, this material is winning the competition in the marketplace and its function reduces the transportation energy and emissions during distribution by extending the sales period.

Another popular source of uncertainty in LCA occurs when the effect of different "scenarios" such as for the "energy" used in the inventory stage is not discussed. It is argued that site-specific energy production data may produce very different conclusions than average or industrial world energy mixes, which are common scenarios used when site-specific data is not available [17]. For example, results may differ when coal-generated electricity data is used for operations that occur in regions in which electricity is produced mainly by hydroelectric power. Coal-generated energy is considered "non-renewable" while hydroelectric power is produced from "renewable" resources and thus appears to be more environmentally friendly.

But perhaps one of the most uncertain parts of any LCA is the impact assessment stage. This is because of an array of reasons. For instance, as described earlier, there is no unified approach for implementation of the impact assessment process. In fact, in ISO words [4]: *"There are no generally accepted methodologies for consistently and accurately associating inventory data with specific potential environmental impacts"*. The two approaches described earlier have their advantages

Table 1. Critical issues at different stages of the LCA process (Based on Huijbregts [14] and Björklund [15]).

	LCA Phase					
			Impact Assessment (LCIA)			
Problem	**Goal and Scope**	**Inventory**	**Choice of Impact Catagories**	**Classification**	**Characterization**	**Weighting**
Data uncertainty		Inaccurate or no emission measurements			Uncertainty in lifetimes of substances	Inaccurate normalization data
Model uncertainty		Linear instead of non-linear modeling	Impact categories are not known	Contribution of impact category is not known	Characterization factors are not known	Weighting criteria are not operational
Uncertainty due to choices (i.e.scenarios)	Choice of functional unit, system boundaries	Choice of allocation methods, technology level	Leaving out known impacts categoires		Using several characterization methods within one category	Using several weighting methods
Temporal variability		Differences in yearly emission inventories			Change of temperature over time	Change of social preferences over time
Spatial variability		Regional differences in emissions inventories			Regional differences in environmental sensitivity	Regional differences in distance to (political) targets
Variability between objects/sources		Differences in emmissions between factories which produce the same product			Differences in human characterisics	Differences in individual preferences, when using the panel method
Mistakes	Any	Any	Any	Any	Any	Any
Estimation of uncertainty		Estimation of uncertainty in inventory parameters			Differences in human characteristics	Estimation of uncertainty in potential impacts

and disadvantages. For instance, since they can aggregate categories under a common basis (e.g. DALYs: Disability Adjusted Life Years), endpoint methods may be preferred over midpoint methods for category weighting, but introduce more subjectivity and uncertainty (e.g. model, scenario and/or parameter) in the assessment since the closer one goes to the end-point categories (i.e. towards the right in Figure 3), the more the models are highly dependent on the user's preferences [18]. Thus, though reconciling efforts are underway within the two main approaches, currently there is no consensus method [10] and comparison studies among these two approaches are often complex to develop and interpret [19,20].

Further, though according to ISO the LCA goal seems straightforward: "LCA is a technique for assessing the 'environmental aspects' and 'potential impacts' associated with a product throughout its whole life cycle", a methodology for studying the general environmental aspects has not even agreed upon. While some consensus has emerged on assessment methods to evaluate contributions to environmental impacts such as climate change, stratospheric ozone depletion, photochemical oxidant formation, acidification and eutrophication [21], the situation is not the same for impact categories such as resource extraction, land use and human health.

LCIA is further challenged by the fact that impact assessment methods rely on models, many of which are being developed or updated to account for current changes in the environment itself. And this stage is further hindered because of the sheer number of chemicals used today. In fact, it has been estimated that of the around 100,000 substances presently used in the world, only about 5% of even the 2000 most used substances have been screened for toxicity and fate [22].

Lastly, several in the LCA community acknowledge that due to the enormous amount of uncertainty involved, product LCIA methodologies based exclusively on mathematical relations representing system flows from and into the environment have important limitations. An alternative is the use of value-based methods, but these in turn need to deal with the open issue of LCIA weighting [23–25]. Tucker [26] offered three alternatives to this LCIA conundrum: use a "reductionalist approach" by reducing the LCIA scope

to obtain a truly robust method; acknowledge the subjectivity of the LCIA method and develop an indicator system that reflects the views of an authoritative forum; or develop an LCIA method that includes the views of different sectors of the society and thus yields results in a more socially acceptable product evaluation.

ISO standards [4–7] recognize the limitations that arise from not effectively addressing uncertainty and variability effectively and urge the practitioner to do so in the LCA study. However, the ISO standards do not suggest any procedure to do so. Ross et al [27] found that out of 30 LCA studies only 3 (10%) included a quantitative or qualitative uncertainty analysis and conclude that the standards need to be revised to ensure that LCA includes at least a qualitative discussion of uncertainty and variability. Among attempts to provide a systematic analysis, Huijbregts [14,28] and Huijbregts et al [29] proposed the first general framework for comprehensive uncertainty and variability evaluation, adding the definition of key concepts in the LCA context. But uncertainty analysis is something new in LCA [30] and thus a number of methodologies for uncertainty estimation have been proposed. Currently, the most favored approach for uncertainty and variability analysis in LCA is incorporating it into the life cycle inventory (LCI) stage. Computer simulation is becoming the method of choice. A number of recent uncertainty and variability methodologies have been proposed and incorporated into LCAs associated with different industry sectors and almost exclusively used Monte Carlo simulation [31–38]. Others use fuzzy logic to perform a similar data evaluation [31,39]. In the packaging sector, despite the increasing use of LCA for evaluating alternatives, a few Monte Carlo uncertainty estimations have been published but with limited background information [37,40–42]. Moreover, although these studies have shown that uncertainty and variability can be incorporated in LCA, the exact implications for decision makers remain unclear.

LCA Enhancements

Enhancements, in the context of this explanation, refer to attempts and approaches to overcome some of the previously stated LCA limitations in order to increase or improve the LCA value, quality and ease of implementation. For the purpose of this discussion, these approaches are grouped into four categories: (a) data-related improvements, (b) streamlined LCA, (c) input-output LCA, and (d) economic analysis and LCA. These areas are some to which increasing research has been devoted in recent years, and it is expected that they will evolve from their current state into more established methods.

Data-Related Improvements

Data related improvements comprise three major aspects that are not necessarily separable: (a) data collection, (b) data availability, and (c) data quality. Regarding data collection, the current status of LCI databases around the world can be used as an indicator of the situation. Worldwide, government, private organizations and research institutes are currently either expanding existing inventories or developing new ones (see Table 2).

Table 2. Summary of LCI databases and managing organizations and their status (updated from Norris and Notten) [44].

	Status	
Managed by	**Completed[1]**	**Planned or under development**
National and Multi-government[2]	Italy, Switzerland (BUWAL 250), Switzerland (Ecoinvent), SAEFL	Australia, Canada, Chinese Taipei, JUapan, Korea, Sweden (SPINE), USA
Consultants and research institutes[3]	Denmark (EDIP), Sweden (CPM), Ecolilan (DEAM)	Austria, Denmark, France, Germany, Sweden, Switzerland, UK, USA
Industrial[4]	IISI, EAA, FEFCO, APME and PWMI, NiDI	
Academic/ Decentralized[5]		Belgium, China, Chile, Estonia, Finland, India, Norway, The Netherlands, Protugal, Poland, South Africa, Spain, Vietnam, Argentina, Malaysia, Thailand

[1]may be updated
[2]Coordinated effort to produce nationally representative and accessible database. Typically involves collaboration between several organizations and some degree of government funding.
[3]Inventories produced by research organizations or consultants and made publicly available in a database, sometimes for a fee (e.g. databases included with LCA software).
[4]Inventories produced and published under the auspices of a particular industry organization. Includes cases where data made only partially available (e.g. for a fee, or only to parties with sufficient motivation for requesting the data). Most often data compiled by consultants, but includes cases where LCI development is done in-house, or by academic or other research organizations.
[5]Includes inventories compiled by academic or other research organizations, made either partially or fully available on an ad-hoc basis (e.g. through journal publications). Countries may have some degree of information sharing (e.g. an LCA society), but no coordinated data gathering effort (i.e. studies are not organized into an accessible database).

Many LCI databases are sector oriented and some already follow ISO 14048 guidelines for data collection. For instance, the U.S. LCI database which is managed by the Athena Institute and hosted by the U.S. National Renewable Energy Laboratory (NREL) [43] is a project to develop publicly available U.S.-based inventories originally focused on the building sector but later expanded to other industry sectors. However, this database has not been peer-reviewed yet and it is not as transparent as it needs to be for clear utilization. Other LCI databases cover several industry sectors and are usually accessible through commercial LCA-specific software developed by consulting companies.

Regarding data availability, attempts have been made to improve the situation. For instance, the SETAC-Europe LCA Working Group on "Data Availability and Data Quality" was formed in 1998, with the goal to focus on the key features of improving the efficiency and quality of data collection [29]. Similarly, NREL in a joint project with the U.S. EPA has developed a global LCI inventory matrix to help centralize multiple-sector LCI databases. The information is obtained by voluntary submission and is categorized according to industry sector and life cycle stage. However, many of the specific LCI databases are accessible for a fee (U.S. EPA, [45]).

Data quality has been the subject of many publications in the LCA literature [29,46–48]. Quality, often defined as "fitness for use", has been used to comprise aspects of the LCA data such as database format, uncertainty, reliability, completeness, age, geographical location and process technology. Thus, approaches to improve quality involve standardization in data collection and processing procedures; data validation by cross-checking of energy and mass balances; use of data quality goals (DQG) and data quality indicators (DQI); use of parameter estimation techniques; use of higher resolution models; critical review; and additional measurements [15]. Each of these approaches has its own disadvantages. For instance, since the development of standardized databases requires consensus among LCA practitioners, it is time and resource demanding. Likewise, making additional measurements of inventory data or using higher resolution models to obtain better estimates are time and resource intensive.

DQGs and DQIs are simpler and more flexible approaches but there is no consensus about their methodologies. In general, DQG is a qualitative scheme to specify the data quality requirements before actual data compilation. DQI can be either a qualitative, quantitative or semi-quantitative technique to assess the quality of already compiled data.

Alternatively, though limited in scope, sensitivity analysis, which is the study of the effect of changes in an independent variable on the LCA outcome, gives more knowledge about the behavior of the model. However, it is time-consuming when not coupled with some kind of data uncertainty importance analysis. In fact, data uncertainty importance analysis is a useful screening methodology that can help concentration on relevant model parameters by establishing some kind of contribution criteria to the overall LCA uncertainty outcome. However, as stated earlier, uncertainty analysis is still a new concept in LCA, and the information in databases required for the analysis (e.g. ranges, standard deviation, probability distributions) is very limited. In fact, publications considering the evaluation of data ranges are scarce. Among the few, Finnveden [48] analyzed the range of the common inventory data in a number of databases and his findings are shown in Table 3. The variation in this table indicates the typical uncertainty of European databases in the mid 1990s.

Streamlined LCA

In practical terms, it can be expensive and time consuming to conduct an LCA according to ISO or SETAC guidelines. Streamlined or abridged LCAs are approaches that are used to obtain timelier and less expensive results. According to SETAC, there are two main kinds of streamlining: approaches within the LCA framework and alternative life-cycle approaches as shown in Figure 4.

Table 3. Expected variations in LCI data [48].

Inventory Parameter	Variation that can be expected
Central, non-substitutable resources	Factor of 2
Less central and substitutable resources	A factor of 10 or more if they are completely substitutable
Outflow that are calculated from inflows (e.g. CO_2)	The same as for the correspondint inflow
Other energy related air emissions	Factor of 10
Other proces-specific emissions	Factor 10–100 or higher if mistakes or very different types of technologies can appear
Total amount of solid waste	Factor of 10
Specific types of solid waste	The variation can be very high partly due to different classification systems in different countries

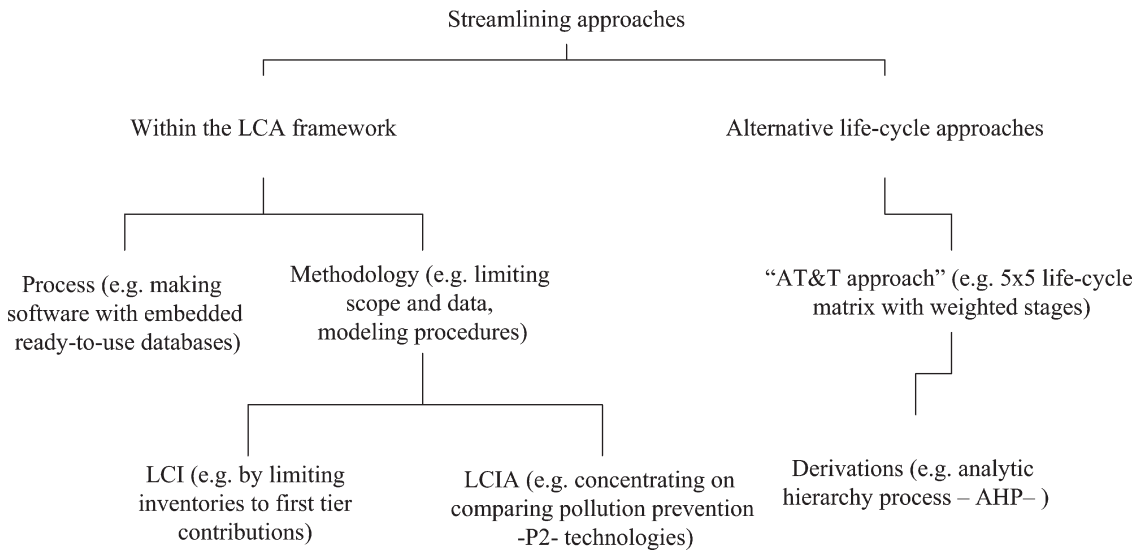

Figure 4. *Streamlining approaches.*

Approaches within the LCA framework can be again subdivided into two: process oriented and methodology oriented. Process oriented streamlining methods deal with the actual operation of performing the LCA (e.g. making software with embedded ready-to-use databases, using process templates, etc.). Methodology oriented streamlining methods deal with simplifying the actual stages of the LCA. By limiting the goal and scope of the study, the LCI and LCIA steps can be simplified. Table 4 contains a list of the common streamlining decisions that have been used in the past, along with their advantages and disadvantages. Since all LCAs are streamlined to some extent, the degree of information "lost" by these techniques cannot be fully accounted for.

Alternative life-cycle approaches do not involve a complete inventory analysis and do not follow the inventory/impact/improvement analysis path required by ISO. Instead, they attempt to evaluate relative differences among alternatives along their life cycles. One of the best examples of this approach is the one developed in 1993 by Graedel and Allenby at AT&T [51] called environmental responsibility product assessment (ERPA) matrix. The ERPA method divides the product life cycle into 5 stages: pre-manufacture, product manufacture, product delivery, use, and recycling or disposal; and considers 5 environmental concerns: material choice, energy use, solid, liquid and gaseous residues. These two dimensions are presented in a matrix format in Table 5.

As indicated in Table 5, each cell of the resulting 5×5 matrix is then assigned a score ranging from 0 (highest impact of a stage on an environmental concern item) to 4 (lowest impact of a stage on an environmental concern item). By this scoring technique, the method estimates the results of more formal LCI and LCIA, and also takes into account whether the possibilities of reducing impacts have been utilized or not [52]. The scores are assigned by consideration of information from actual life cycle studies, checklists, manufacturing surveys and experience. The ratings in a matrix can be added up (i.e. to sum up to a maximum of 100) or can be plotted in a circumference target plot for more convenient evaluation (e.g. center or zero represents highest impact, circumference represents lowest impact). This technique, which critics say is subjective, was found useful for identifying hot spots and opportunities for environmental improvement and has been used recently for a number of different product categories and applications range from re-refined oil in Japan [53], to evaluating the environmental impact of a residential refrigeration unit with a proposed maintenance and take back service [54].

Input-Output LCA

In conventional (SETAC/ISO) LCAs, the system boundary is usually chosen with the assumption that addition of successive upstream production stages has a small effect on the total inventory. However, truncation errors inherent to conventional LCAs have been estimated and in cases can be of the order of 50% [55]. One way to address the boundary issue in LCA is by using economic input-output methods. Economic input-out-

Table 4. Methodology-oriented streamlining approaches (based on Todd and Curran [49], and Hunt et al [50]).

Streamlining approach	Application procedure	Advantages	Cautions
Removing upstream components	All processes prior to final material manufacture are excluded. Includes fabrication into finished product, consumer use, and post-consumer waste management.	Clear boundaries set. All the products and processes directly involved in producing the product are considered. Eliminates proprietary vendor data issue.	Important environmental consequences of raw material extraction or production may be eliminated from consideration, causing a skewed result.
Partially removing upstream components	All processes prior to final material manufacture are excluded, with the exception of the step just preceding final material manufacture. Includes raw materials extraction and precombustion processes for fuels used to extract raw materials.		
Removing downstream components	All processes after final material manufacture are excluded.	Captures some important environmental concerns within the life cycle useful in product improvement. Results can be used to support environmental procurement programs.	Ignores important stages in the life cycle. For example, the use stage for some products (e.g. building materials), final disposal (e.g. packaging).
Removing up- and downstream components	Only primary material manufacture is included, as well as any precombustion processes for fuels used in manufacturing. Sometimes referred to as a "gate-to-gate" analysis.	Data gathered and processes under the study can be directly affected by user. Results likely to be useful to sponsor.	Actual life cycle of material is missed.
Using specific entries to represent impacts	Selected entries are used to approximate results in each of 24 impact categories, based on mass and subjective decisions; other entries within each category are excluded.	Focuses on environmental considerations deemed important by the user. Helpful when regional considerations are of critical importance.	Other important environmental considerations can be excluded, thus results-based decisions may not be the best for the environment or human health.
Using specific entries to represent LCI	Specific entries from the individual processes comprising the LCI that correlate highly with full LCI results are searched for; other entries are excluded.	Selected surrogate inventory entries may be useful to evaluate potential "what ifs" scenarios.	Surrogates must be carefully chosen to ensure that surrogate truly represents the product, material, or process under study.
Using " showstoppers" or "knockout criteria"	Criteria are established that, if encountered during the study, can result in an immediate decision.	Focuses on specific issues deemed important by the user. No need to explore effects of all constituents.	Other important environmental considerations can be excluded, thus results-based decisions may not be the best for the environment or human health.

(continued)

Table 4 (continued). Methodology-oriented streamlining approaches (based on Todd and Curran [49], and Hunt et al [50]).

Streamlining approach	Application procedure	Advantages	Cautions
Using qualitative or less accurate data	Only dominant values within each of 6 process groups (raw materials acquisition, intermediate material manufacture, primary material and product manufacture, consumer use, waste management, and ancillary materials) are used; other values are excluded, as are areas where data can be qualitative, or otherwise of high uncertainty.	All potential environmental issues are detected at each stage of the life cycle. Some environmental factors not readily amenable to quantification (e.g.biodiversity, habitat issues), can be considered.	Difficulty in assessing importance of each environmental concern in overall life cycle and in comparison to other products.
Using surrogate process data	Selected processes are replaced with apparently similar processes based on physical, chemical, or functional similarity to the datasets being replaced.	Estimates can be developed for data that would otherwise be unavailable.	Surrogates must be carefully chosen to ensure that surrogate truly represents the product, material, or process under the study.
Limiting raw materials	Raw materials comprising less than 10% by mass of the LCI totals are excluded. A 30% limit has also been used.	Limits the number of items and focuses on those that are likely to be most important for the product under study. Easy to define clearly and does not have an inherent bias.	By focusing only on volume and disregarding hazard or toxicity, important environmental effects may be overlooked.

Table 5. Environmentally responsible product assessment matrix [51].

	Environmental Concern				
Life cycle stage	**Material choice**	**Energy use**	**Solid residues**	**Liquid residues**	**Gaseous residues**
Premanufacture (i.e., raw material extraction/production)	(1,1)	(1,2)	(1,3)	(1,4)	(1,5)
Product manufacture	(2,1)	(2,2)	(2,3)	(2,4)	(2,5)
Product delivery	(3,1)	(3,2)	(3,3)	(3,4)	(3,5)
Product use	(4,1)	(4,2)	(4,3)	(4,4)	(4,5)
Recycling, disposal	(5,1)	(5,2)	(5,3)	(5,4)	(5,5)

put LCA (EIO-LCA) is the result of applying economic input-output (EIO) analysis to help perform a life cycle assessment. The EIO analysis is based on using the EIO matrices that are regularly estimated for most developed countries and economies. An EIO matrix is a transaction matrix that shows the relationship between the different sectors that form part of an economy. Table 6 shows the basic structure of such a matrix in which entries are expressed in dollars. For example, a12 is the amount of dollars required to (directly and indirectly) input in sector 1 (e.g. electricity) to obtain $1 worth of sector 2 (e.g. aluminum sheeting) output. For the U.S., the EIO matrix has 519 sectors, so it is a 519 × 519 matrix.

The basic approach for EIO-LCA then can be compactly summarized using matrix algebra notation in equations 1 and 2 as described by Lave and colleagues [56,57]:

$$\mathbf{X} = (\mathbf{I} - \mathbf{D})^{-1}\,\mathbf{F} \tag{1}$$

$$\mathbf{B} = \mathbf{R}\mathbf{X} \tag{2}$$

In Equation (1), **X** is a vector containing the total output (in dollars) from different sectors of the economy required to meet a desired final demand, **I** is a identity matrix (i.e. to include the output of the aluminum sheeting sector itself), **D** is the EIO matrix, and **F** is a vector representing the desired final demand (e.g. dollars worth of a desired amount of aluminum sheets). In Equation (2), **B** is the vector containing the economywide environmental burdens (e.g. toxic emissions or electricity use), and **R** is a matrix representing the environmental burden per dollar output of each sector.

Table 6. Basic structure of an EIO matrix.

	Output			
Input	**Sector 1**	**Sector 2**	…	**Sector *n***
Sector 1	a_{11}	a_{12}	…	a_{1n}
Sector 2	a_{21}	a_{22}	…	a_{2n}
⋮	⋮	⋮		⋮
Sector *n*	a_{n1}	a_{n2}	…	a_{nn}

By setting the boundary of the LCA on the level of the national economy, EIO-LCA with the EIO matrix attempts to address the boundary issue including the interdependence of different processes. However, this analysis has its own problems including the high level of aggregation (i.e. combination of product and technology information) in industry or commodity classifications, which limits the level of detail of EIO-LCA studies. Moreover, there is incompleteness of sector-based environmental statistics, which in turn limits the accuracy of the EIO-LCA results.

Because of these reasons, conventional LCA is often seen as more detail oriented. However, these analyses are more labor- and time-intensive, and suffer from the stated truncation error (i.e. due to omission of contributions outside its finite boundary). In fact, due to the quick and inexpensive nature of the EIO-LCA approach developed by researchers at Carnegie Mellon University, Matthews and Lave [58] suggested the use of this system to help in corporate benchmarking efforts to evaluate the environmental performance of their operations. Moreover, using input-output techniques, Suh [59], from Leiden University has recently developed the MIET, an inventory estimation tool for missing flows.

"Hybrid" analyses combine process-level data with sector-level input-output analysis and thus try to get the best of both approaches. According to Suh et al [60], hybrid approaches can be grouped into three different categories, which are tiered hybrid analysis, input-output based hybrid analysis, and integrated hybrid analysis. Table 7 summarizes the main aspects of each approach along with their perceived advantages and disadvantages.

Table 7. Main hybrid approaches combining process-based analysis and input-output based analysis (based on Suh et al [60]).

Approach	Characteristics	Advantage	Disadvantage
Tiered hybrid analysis	Direct and downstream requirements (e.g., construction, use, maintenance, and end-of-life) and some important lower order upstream requirements of the product life cycle are examined in a detailed process analysis. Remaining higher order requirements (e.g., materials extraction and manufacturing of raw materials) are covered by input-output analysis. Exact location and comparability of the boundary between the process and input-output analysis part depends on data availability, desired detail and accuracy, and constraints in terms of cost, labor, and time.	Easy to use. May be useful to address dependency upon imports.	Issues with double counting. Issues with reccurring flows (e.g. recycling, reuse) in process-based analysis part
Input-output based hybrid analysis	Major input-output sectors are further disaggregated in case more detailed sectoral monetary data are available. Disaggregation may reach a resolution of the level of process-specific studies.	Consistent method Avoid; double counting.	Use and end-of-life phase are externally added Issues with recycling flows (e.g. recycling, reuse) between use and end-of-life stages and input-output part.. Should be combined with other method; if national economy is highly dependent upon imports.
Integrated hybrid analysis	The process-based system is represented in a technology matrix by physical units per unit operation time of each process while the input-output system is represented by monetary units. This model links the process-based and the input-output-based systems through flows crossing the border.	Consistent matrix framework for the whole life cycle. Avoids double counting. Easy to apply analytical tools.	Complex to use. Time- and data intensive.

The connection between process-based and input-output-based LCA is a topic under development [61] and thus much research work still needs to be done to define this relationship. Nevertheless, recent assessment studies have already benefited from the information provided by these hybrid analyses. For example, by using a hybrid approach, Norris et al [62] were able to estimate the energy consumption during the factory-to-mall phase of life cycles that has been universally neglected in process-style LCAs; and Nakamura and Kondo [63] have developed a hybrid approach that expanded the input-output system to include waste flows and showed that the EIO model is in fact a special case of their model.

Economic Analysis and LCA

While LCA can be useful for evaluating environmental attributes of a system, it is often criticized for not providing monetary information that business managers routinely need to allocate the often scarce capital resources available to minimize the environmental footprint of their business operations. Thus, various approaches have been developed to supplement environmental information with cost information and enhance the decision-making process. The central challenge is estimating of the "environmental cost" of business operations and a whole body of concepts and terms has been developed under the umbrella of "environmental accounting" to address this issue (U.S. EPA, [64,65]).

The scope of the present discussion comprises life cycle based approaches to estimate environmental costs of products. One of these approaches is life cycle costing (LCC). LCC is a systematic procedure for identifying environmental consequences along the life cycle of a product (i.e. product line, process, system or facility), and assigning measures of monetary value to those consequences using accounting procedures. This process includes the assessment of material flows (e.g. amount of solid waste generated) through the product system (i.e. materials accounting, essentially a kind of LCI) as well as costs (i.e. cost accounting), including environmental costs (e.g. waste disposal).

Despite the apparent compatibility of approaches, LCA and LCC have important methodological differences. For example, while LCA attempts to evaluate the relative environmental impact (from a broad societal

Table 8. Differences between LCA and LCC [66].

Items	Tools	
	LCA	LCC
Objective	Compare relative environnental performance of alternative product systems for meeting the same end-use function, from a broad, societal perspective	Determine cost effectiveness of alternative investment and business decisions, from the perspective of an economic decision maker such as manufacturing firm or a consumer
Scope of life cycle	Supply chain of processes supporting usage phase; entire physical usage	Activities directly causing costs or benefits to the decision maker during the economic life ofthe investment as a result of the investment
Flows considered	Pollutants, resources, and interprocess flows of materials and energy	Direct costs and benefits to decision maker
Units for tracking flows	Physical and energy units	Monetary units (e.g. dollars)
Time treatment and scope	Timing ignored; all causally linked flows, and some of their impacts collapsed in time and valued equally regardless of timing	Timing is critical; present valuing (discounting) of costs and benefits; specific time-horizon scope, outside of which costs and benefits are ignored.

perspective) of alternative product systems that perform the same function, LCC intends to estimate the relative cost effectiveness of alternative investments and business decisions, very often from a private perspective. Thus, LCA and LCC actually consider life cycles with different spans and flows (i.e. physical or energy units vs. monetary units) that are not necessarily compatible. In a succinct table (Table 8), Norris [66,67] summarizes the extent of the differences between life cycle assessment and life cycle costing methodologies. Furthermore, the LCC outcome is limited when used at the product design stage (e.g. Design for the Environment programs), since it suffers from greater uncertainty than LCA [68]. This is because future technological changes have a strong effect on the results, and because of specific additional factors (e.g. interest rate and market dynamics) that are not always stable and are independent from technology changes.

However, by offering direct opportunities for cost reduction, LCC is perceived to help to promote life cycle based analysis. In turn, economic analysis with a life-cycle perspective has the potential of discovering "hidden" costs (see Table 9, cost types 2, 3, 4 and 5) and revenue impacts that are otherwise neglected in conventional economic analyses. Very often, though, LCC users utilize the pragmatic approach of focusing exclusively on internal or "private costs" (i.e. type 1 and some type 2). Just recently, some comprehensive approaches have been developed to bridge the gap between LCA and LCC and to improve it to allow for easier identification of hidden costs. For instance, Total Cost Assessment [69], method developed by a joint effort of private companies and the American Institute of Chemical Engineers' Center for Waste Reduction Technologies, is an approach that facilitates the inclusion of environmental costs into a capital budgeting analysis by classifying costs into categories shown in Table 9.

Several alternative versions of life cycle costing methodologies have also been developed, mostly by interested companies, and confusion still exists about the concepts, scope and terminology involved [69,70].

LCA and Packaging

Packaging situations were one of the earliest applica-

Table 9. Categories of costs according to AICHE/CWRT (Center for Waste Reduction Technologies-AIChe, [69]).

Cost Type	Description
Type 1 : direct	Direct costs of capital investment, labor, raw material, and waste disposal. May include both recurring and nonrecurring costs. Includes both capital and operations and maintenance (O&M) costs.
Type 2: Indirect	Indirect costs not allocated to the product or process (overhead). May include both recurring and nonrecurring costs. Includes both capital and O&M costs.
Type 3: Contingent	Contingent costs such as fines and penalties, costs afforced cleanup, personal injury liabilities, and property damage liabilities.
Type 4: Intangible	Difficult to measure costs, including consumer acceptance, customer loyalty, worker morale, union relations, worker wellness, corporate image, and community relations.
Type 5: External	Costs borne by parties other than the company (e.g., society).

tions of LCA. Harry E. Teasley, Jr., manager of the packaging operations at the Coca Cola Company was credited for first devising the analytical scheme of quantification of material, energy and the environmental burdens of a package over its complete life cycle from raw material to disposal in 1969 [71]. About three decades ago, public concern over increasing volumes of solid waste due to the use of plastic in packaging and later concerns about energy consumption became the major driving forces to study the effects of packaging on the environment [72]. A representative study is the comprehensive energy analysis of production and use of packaging systems published by Boustead and Hancock [73]. Eventually, these studies evolved into the comprehensive tool that LCA is today and example studies are those such as that published by the Swiss Agency for the Environment, Forests and Landscape (SAEFL) [74,75] and the series of "eco-profiles" of plastic resins and intermediates, conversion processes, and packaging published by the Association of Plastics Manufacturers in Europe (APME) [76–85].

Applications of LCA in packaging situations can be divided into two main categories depending on which constituencies use it, namely: a) stakeholder, and b) third-party organizations.

a) Stakeholder Use of LCA

A stakeholder is any interested (often private) body that might use LCA, or some form of LCA, in decision making regarding product design, product improvement, product comparison, strategic planning, compliance with regulatory policy, marketing, or academic purposes, for example.

Recent examples of the use of LCA for product development and improvement purposes can be drawn from several industries. Most of them resulted from corporate environmental stewardship programs that generally involve proactive premises such as design-for-the environment (DfE). DfE intends to integrate health and environmental considerations into business decisions and it has been applied in Europe as well as in the U.S. While in Europe, most DfE programs are voluntary and often internally adopted by companies that want to comply with the strict disposal regulations in place, in the U.S. DfE is mostly known as a voluntary partnership between the U.S. Environmental Protection Agency and industries to attempt to help with pollution prevention [86]. DfE principles, along with integrating health risks, aim to use "accepted" results from LCA studies to create the attractive but unproven concept of an "environmental preference ranking" or "environmental indices" for the selection of materials to be used when designing new products [87, Industrial Designers Society of America, [88]). The Cleaner Technology Substitute Assessment (CTSA) methodology developed by the EPA, which involves comparative evaluation of substitute technologies, processes, products or materials, regarding human health, environmental risk, performance, cost and resource conservation, is another tool (along with LCA) that is used under the DfE premise [89] to try to help with process selection. Many DfE programs are internally developed by companies that often claim substantial economic benefits after implementing DfE-recommended improvements. For instance, Xerox Europe, using DfE under its "waste-free initiative", reported utility savings by pushing towards the reuse and recycling of equipment components through appropriate labeling and improving disassembly, as well as the reuse of packaging components by reducing the number of pallet styles and boxes used for new equipment, and by switching from conventional single-use corrugated boxes to wooden and steel totes for the collection of used equipment [90]. Likewise, U.S. examples exist on the use of LCA for product improvement purposes by studying packaging options. For instance, an LCA conducted to evaluate the environmental performance of the yogurt product delivery system used by Stonyfield Farm Inc. [91,92] analyzing different packaging formats (i.e. 4, 6, 8 and 32 oz polypropylene cups and 2 oz linear low-density polyethylene), estimated that the greatest potential improvements were the redesigning of the primary packaging and the use of alternative manufacturing techniques for the yogurt cups. The study indicated that in this case, shifting from injection molding to thermoforming of 32 oz containers reduced the life cycle energy by 18.6% and solid waste by 19.5%, primarily due to light-weighting. The authors claimed that elimination of overcaps for 6 oz and 8 oz containers provided similar advantages, and indicated that the effect of container size was significant when it was found that delivering yogurt in 32 oz instead of 6 oz containers could save 14.5% of the life cycle energy and decrease solid waste by 27.2%.

Along with product improvement applications, partnership of industry with research institutions using packaging related "case studies" attempted to evaluate the environmental aspects of a number of industry operations [91–95] as well as the LCA methodology itself. For instance, researchers from the University of Mel-

bourne used a case study involving the utilization of different packaging formats by an Australian-based maker of refrigerators, to estimate the effects of excluding and including site-specific data [94]. By limiting their analysis to a single non-global cumulative impact category such as the presence of significant photochemical precursors in the atmosphere, they reported the ability to assess whether an improvement in protective packaging produced any noticeable change in this impact category under two scenarios (i.e. when aggregated into a single global parameter or when spatial and temporal factors were taken into account).

LCA has been often used for product comparison purposes as well, but due to its nature, the assessment shows the environmental implications of different choices and the trade-offs that need to be made, instead of a clear answer. Nevertheless, oftentimes the complexity in the interpretation of the results is overlooked in many LCA comparison reports that, deliberately or not, portray one alternative as more environmentally sound than the other.

A survey of the literature in order to attempt an analysis of the use of LCA in packaging comparisons was presented by Martino [96] and is shown in Table 10. The table presents a list of some packaging oriented LCA and LCI involving comparison studies which have been released or published in peer-reviewed journals, summary reports or books, in the last fifteen years. It can be seen from the Table that, regardless of the packaging formats evaluated in the studies, their scope is relative consistent comprising processes from raw material extraction to, in most cases, disposal. Likewise, in most cases, the functional units have been selected comprising the containment of the product and in some instances including its delivery to the consumer.

Consistency is also noticed with regards to the inventory parameters/impact assessment indicators since all of them include energy used (though many don't indicate whether it is a gross or net value), and some include warming emissions. In some instances the impact assessment indicators belong to a pre-established set based on a particular method (e.g. SimaPro).

Not surprisingly, the consistency diminishes when analyzing, the data sources and the types of scenarios considered, since they are directly related to the systems studied and the selected functional units. In general, in only one instance an uncertainty analysis has been included and its results included in the conclusion. It can also be noticed that when such studies are used for marketing purposes, their conclusions seem less qualified and more absolute and there is no critical review included.

Another popular use of LCA in packaging is when LCA has been used from a waste management perspective to attempt to identify environmental burdens of a certain waste management operation, or to determine what is the environmentally better waste treatment system for packaging materials [107–110]. Within the waste management programs often studied by LCA, often included are take-back programs, which are taking root in a number of industries (i.e. automobiles, computers and other electronic devices, adhesives and garments) for dealing with the disposal of their products. Product take-back programs and "extended producer responsibility" (EPR), have become popular in many countries [111–113]. The take-back systems are founded on the idea of product "recovery" by the manufacturer or "reverse logistics" and by involving appropriate planning, managing, and optimizing of the forward as well as reverse distribution streams of both new and used products. They have to be not only cost-effective, but also to reduce the environmental footprint of an operation, but not necessarily by packaging reuse. While a number of these programs have developed as the result of legislation, some of these programs are voluntary, set by industries. For instance, the electronic components industry, due to its high volume of bulk shipments to a relatively small number of globally distributed locations and the near pristine condition of the packaging used after shipment, seems to be especially suitable for instituting packaging take-back systems [114,115]. Furthermore, these take-back systems may be part of broader initiatives such as DfE.

b) Third party use of LCA

A third party, in this case, is any independent (governmental or private) body that might use LCA, or some form of LCA, for either of two purposes: 1) help with environmental labeling programs, or 2) help with policy-making.

Environmental labeling programs

By a third party, LCA can be used to help obtain information required by some environmental labeling schemes. For most developed (and some developing) countries, environmental labeling in general comprises more or less the types of environmental labels listed in Figure 5.

In recent years, steps for harmonizing and standardizing environmental labeling programs worldwide

Table 10. List of selected packaging oriented LCA and LCI involving comparison studies.

Product	Packaging formats and materials compared	LCA type/Data source	Main Application	Scope highlights	Scenarios considered	Functional unit/basis for comparison	Inventory/ Impact indicators considered	Summary of conclusions	Critical review	Year and reference information
Hot drink	Single use polystyrene (PS) and paper cups	Process based/Public databases date around 1981	Case study	Extraction of raw materials, paper and plastic manufacture, use and disposal (i.e. landfilling and incineration). Recyling of packaging that is not disposed.	N/a	Same size of hot drink cup for fast food applications	Raw material and utility (i.e. steam, cooling water, power) use, air and water emissions.	PS cups consume less raw materials, utilities. Produce less air and water emissions with the exception of some alkane emission.	Yes	Hocking; Wells; McCubbin; Cavaney; Cammo, 1991 [97,98]
Pallet load	Polyethylene (PE) stretch wrap and a glue-like unitizing system (i.e. Lock'n Pop®).	Process based/private and public databases dated around . . .	Marketing	Extraction of raw materials, processing, manufacturing, use and disposal (i.e. incineration and landfill). Incineration is credited.	N/a	Unitization of 1625 model pallet loads	Energy, oil and landfill use, carbon dioxide and water emissions.	Lock'n Pop® use less energy and oil, produce less emissions and wastes.		Dumbleton Consulting, 1992 [99]
Soft drink	Like-sized containers: polyethylene terephthalate (PET) (16-ounce), glass (16-ounce) and aluminum (12-ounce) soft drink containers.	Process based/Private database	Marketing	Extraction of raw materials, processing, manufacturing and filling of primary containers to secondary packaging and distribution. Disposal burdens of material that is not recycled. Incineration is credited.	1995 reclycing rates	1000 gallons of soft drink purchased by consumer	Energy, Waterbone and airborne emissions, solid waste	PET consumes as less energy than one-way glass and as much energy as aluminum. PET produces less waterborne and airborne emissions, less waste		Franklin Associates for the National Association for Plastic Container Recovery, 1995 [100]
Beer	One way and returnable glass bottles	Process based/ Private database (Belgium brewery)	Case study	Production and transport of bottles to filling, production of secondary and tertiary packaging and transport to distributor. Energy for washing and transporting returned bottles, Transport and replacement by new bottles plus transport and recycling of the bottles that are removed from the system.	15% glass recycling rate. Different break rates, transport distances, truck tonnage	Packaging and delivery of 1000 l of beer in bottles of 25 cl	Energy	Regardless of transport distance, when break rate is <5% returnable glass consumes less energy than one way glass bottles	No	Van Doorselaer and Lox, 1999 [101]
N/a	Nylon 66, Nylon 6, polycarbonate (PC), low density polyethylene (LDPE), polypropylene (PP), high impact polystyrene (HIPS), general purpose polystyrene (GPPS), polyethylene terephthalate (PET) and polylactide (PLA) resin material	Process based/Private and APME databases, own estimations.	Product development/improvement	Craddle to gate study. Raw material extraction and resin manufacture of PLA. Values for petrochemical polymers were obtained from related APME studies.	PLA generation I and II polymerization processes, combined with energy source alternatives such as biorefinery public electricity grid and wind power	1 Kg of polymer	Gross energy, global warming emissions, fossil fuel and water use	PLA resin uses less gross energy and produces less GWP emissions than the rest. PLA is surpassed only by PET in terms of least water consumption.	No	Vink et al, 2003 [102]

(continued)

Table 10 (continued). List of selected packaging oriented LCA and LCI involving comparison studies.

Product	Packaging formats and materials compared	LCA type/Data source	Main Application	Scope highlights	Scenarios considered	Functional unit/basis for comparison	Inventory/ Impact indicators considered	Summary of conclusions	Critical review	Year and reference information
Yogurt	8oz polylactide (PLA) and polypropylene (PP) thermoformed cups	Process based/Private database	Case study	Extraction of raw materials, container manufacturing, use, disposal (i.e. landfilling). Recyling of product that is not disposed.	Double and triple effect evaporation for aquous lactic acid distillation. Landfill biodegradation of PLA to methane with methane collection and combustion. No landfill biodegradation of PLA.	1000 kg of yogurt purchased by consumer	Global warming emissions and gross energy	Thermoformed PLA cups consume less energy than PP cups as long as triple effect evaporation is used for lactic acid recovery. Difference is within margin of error when double effect evaporation is used. PLA and PP greenhouse gas emissions from landfill are equivalent provided PLA does not biodegrade. Otherwise PLA greenhouse emissions are higher than PP's.		Bohlman, 2004 [103]
Yogurt containers	Conventional wooden pallet and a specific-purpose bulk packaging system (i.e. Enviropak® T760).	Process based/APME, RMIT and SimaPro software	Case study	Manufacture of raw materials into primary materials (e.g. resins, timber, etc), packaging manufacturing, transport, use, recycling and disposal of the two packaging systems in New Zealand, Excluded upstream processes, (i.e. extraction of raw materials, manufacture and maintenance of equipment).	Uncertainty information about weighting factors included in analysis.	A unit of the plastic packaging system (i.e. Enviropak® T760) and a wooden pallet.	Impacts included in the Environmental Priority Strategy 2000 Default method.	The Enviropak® T760 obtained a better score than the wooden pallet across the impacts considered in the EPS 2000 Default method.		Lee and Xu, 2004 [104]
Soft drinks and mineral water	1.5 l one-way polyethylene terephthalate (PET) and 0.7 l refillable glass bottles. Other sizes are also included.	Process based/Not specified in source surveyed	Marketing	Extraction or raw materials, container manufacturing, use, disposal (i.e. incineration and landfill). Recycling of product that is not disposed using the expanded boundaries approach.	German kerbside collection and recycling system (Germany) and deposit based recycling system (Far East).	1000 l of beverage	Global warming emissions, fossil resource, acidification, terrestrial and acuatic eutrophication, smog, use of nature.	Environmental impact differences between the 1.5 l one-way PET bottles and the reusable 0.7 l glass bottles are within margin of error of the study when both are recycled within the German kerbside system. When PET bottles are recycled outside German system, the difference is notable.		Institute for Energy and Environmental Research (IFEU) in Heidelberg, Germany for the PET Container Recycling Europe, 2004 [42]

(continued)

Table 10 (continued). List of selected packaging oriented LCA and LCI involving comparison studies.

Product	Packaging formats and materials compared	LCA type/Data source	Main Application	Scope highlights	Scenarios considered	Functional unit/basis for comparison	Inventory/Impact indicators considered	Summary of conclusions	Critical review	Year and reference information
Mail-in-order soft goods	Corrugated boxes with various types of dunnage and shipping bags composed of paper and/or plastic.	Process based	Purchasing analysis	Raw material extraction, packaging manufacture, transportation to order preparing facility, transportation to customer, disposal. Recycling and reuse also considered.	Specific packaging systems included more than one material (plastic, paperboard, paper, etc). Most packaging components were analyzed under two levels of recycled content.	10,000 arbitrary (i.e. 17.5″ x 12″ x 2.5″—uncompressed height- and a weight of 1.28 pounds) packages of soft goods items to customers	Energy use, air and water emissions, solid waste.	Weight of packaging is the most critical factor influencing environmental indicators. For example, box systems which were more than four times heavier than bags required more production energy. Boxes twice as heavy as bags produced more waste and greenhouse gas emissions.	Yes	Franklin Associates for the Oregon Department of Environmental Quality and the U.S. EPA, 2004 [105]
Tape recorder	Expanded polystyrene and corrugated paperboard inserts	Process based/European Manufacturers of EPS packaging (EUMEPS), European Database for Corrugated Paperboard Life Cycle Studies, APME and journal publications.	Case study	Raw material extraction, insert manufacturing, assembly, transportation , use and transportation and disposal of packaging materials.	Redesigns of inserts using less material weights. Different end-of-life scenarios (i.e. landfilling and incineration rates) in Siingapore	Same internal protective function of holding a tape recorder securely in a box.	Impacts included in SimaPro LCA Version 5.0 software's Eco-indicator 99 method: climate change (global warming emissions), acidification /eutrophication, ecotoxicity, fossil fuels and respiratory inorganics.	Both redesigned inserts obtained better scores than original ones across the impacts considered by Eco-indicator 99. Higher rates of incineration, as opposed to landfilling, resulted in better Eco-indicator 99 scores.		Tan and Khoo, 2005 [106]

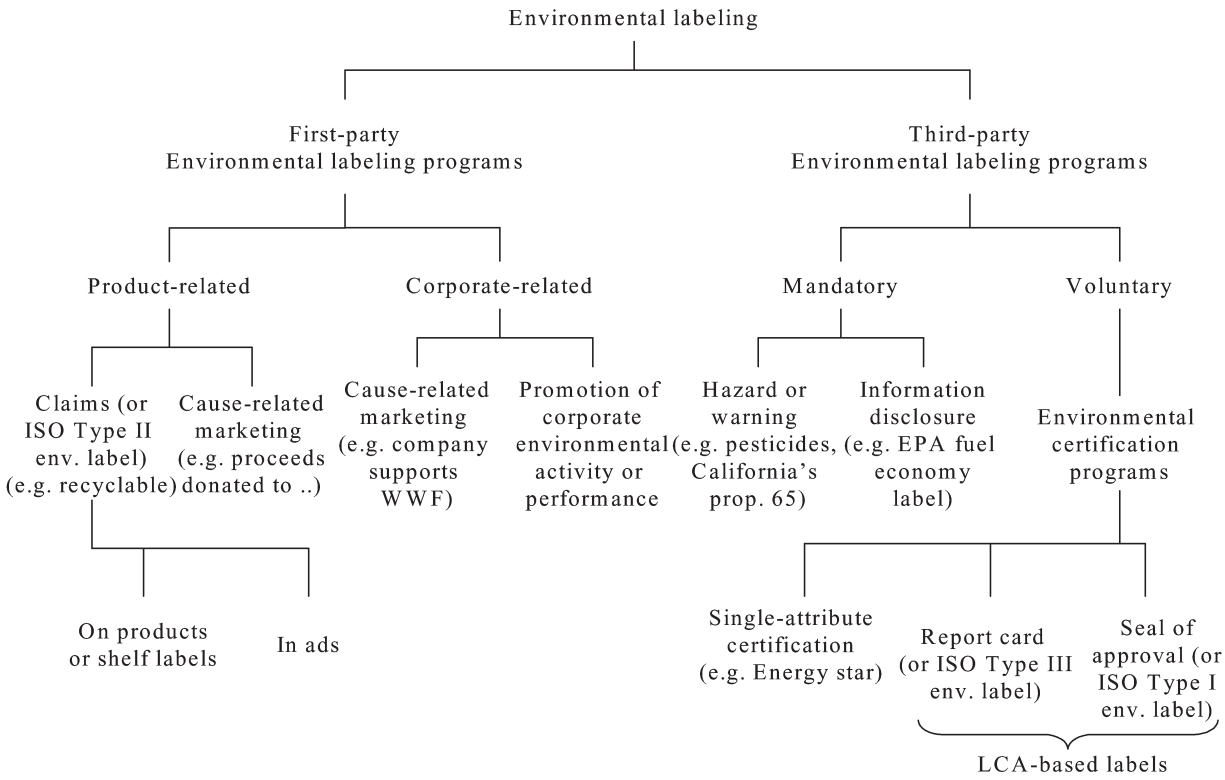

Figure 5. *Environmental label classification (based on Environmental Labeling Issues, Policies and Practices Worldwide, United States Environmental Protection Agency [119]).*

have been taken by ISO, with the release of a set of standards in which it recognizes three types of voluntary environmental labels: Type I (Environmental labels and declarations), Type II (Self-declared environmental claims) and Type III (Technical report-environmental labels and declarations). ISO Type II labels, also called "green claims" or "green symbols" are issued by the interested party which itself creates the label, applies the label, and establishes controls to ensure that its product meets the claims on the label [116]. In some cases, these claims may be certified under single-attribute third-party certification programs. Green claims are the most widely used environmental labels [117] and are not based on product life-cycle concepts. Instead, they are general statements about whether a product is recyclable, contains recyclable material, is degradable/biodegradable/photodegradable, or compostable or source reduced, or refillable, or ozone safe, etc. In countries with regulations that allow the use of this type of labels, claims are required to be accurate and not misleading in order to comply with national legislation and trade regulations. For example, in the U.S., federal (Federal Trade Commission) as well as state bodies have developed guidelines to regulate such claims [118].

Though the purpose of these claims is to provide the consumer with accurate information about positive environmental attributes of products and to help with international trade, a couple of issues arise with their use. First, due to economic limitations it is very difficult for an average consumer to challenge or question these statements since tests are often expensive and time consuming. Second, these claims often focus on one stage of the product life cycle, disregarding other stages that potentially may be more harmful to the environment, and thus providing misleading information. In fact, Lavallée and Plouffe [117] argue that the widespread use of such labels has hurt the development of LCA-based labels.

Type I and Type III labels are issued by a third party and involve LCA-based analysis for the certification. Type I labels, also known as seal-of-approval, are the outcome of what are usually called eco-labeling programs in which a product, process, or management system is certified to meet specific environmental criteria as established by a third party organization. Oftentimes, manufacturers make prior use of LCA under programs such as DfE that help environmental stewardship, to "self-certify" their processes before third-party valida-

tion. The third party can be the owner or administrator of the label program, which usually has three basic steps: (a) selection of product category (e.g. by similar function, and/or similar environmental impacts, and/or importance of product in marketplace); (b) development of requirements to be met (e.g. by using some form of LCA along with peer-review process, selection of the system's most relevant contributions to environmental impacts and guidelines for their reduction are set); and (c) certification and licensing (i.e. compliance verification and testing, applicant licensing and monitoring). Worldwide, eco-label programs vary on how they are run or sponsored. They can be administered by governments, private companies (for profit and non-profit), non-governmental organizations, or some combination of the above.

Though both ISO Type I and Type III environmental labels involve LCA concepts, they differ with respect to the way they convey the information. By assuming that the information from LCA results is too complex and too extensive to present on a label, Type I label programs first decide which stages of the LCA are the most significant for the determination and weighting of the certification criteria, and finally evaluate and issue the seal-of-approval for qualifying products. Alternatively, ISO Type III labels aim at presenting to the consumer much more detailed environmental information, including items such as energy use and environmental impacts in a report-card format, and assume that consumers can themselves prioritize across environmental burden categories and thus themselves do the judgment.

Several issues stir debate and complicate the use and implementation of these environmental labeling programs. For example, while ISO standards require an LCA compliant with ISO 14040, in-depth LCAs are seldom used for awarding these labels because they are cost- and time-intensive. Instead, these programs end up considering only certain stages of the life cycle [119], usually by extrapolating from environmental performance results of similar products offered on the market. Furthermore, regarding type III labels, since the selection of labeling criteria is not based on the same LCA methodology, product comparisons cannot be made, thus confusing the consumer at the moment of judging the preference.

Issues may also occur due to the complexities added by global economic trends, trade agreements and logistical practices when considering imported goods and the environmental assessment of their life cycle [120]. Further, due to the nature of the ecolabeling programs and their LCA-based approach, often the programs end up awarding preferability seals to products made with state-of-the-art technologies that are difficult to obtain in less developed regions or countries, thus creating animosity towards the results of these studies (World Trade Organization, [121]).

Standardization efforts, though very costly and/or technically challenging in some cases, have been made in order to achieve harmonization and/or mutual recognition among programs. In fact in 1994, national and multinational ecolabel licensing programs founded the Global Ecolabeling Network (GEN) with the objective to "improve, promote and develop the ecolabeling of products" (Global Ecolabeling Network, [122]). Currently GEN has twenty-six members with programs such as the well-established Green Seal (U.S.), Blue Angel (Germany), TerraChoice (Canada), European eco-label (E.U.) and Eco Mark (Japan).

Policymaking

The use of LCA for policy making is a practice that sometimes faces strong opposition from trade and industry organizations and even from countries. So far, the governments within the European Union have the most experience in using LCA concepts for developing policies.

The Integrated Product Policy (IPP) developed in 1999 by the European Commission, is a product-oriented approach to government policy that attempts to reduce environmental degradation by addressing all phases of a product's life cycle. The IPP approach uses a number of instruments, such as economic assessments, product stewardship programs, substance bans, voluntary agreements, environmental labeling and product design guidelines, to address the system life cycle impacts of products and processes.

Not surprisingly, though, actual LCA-based policy making, as for any other type of environmental policy, has many critics, and may have economically sensitive consequences for many industries when policymakers consider taxing or restricting what are found to be environmentally unsound products (Europen, [123], Europen, [124]). For example, many European countries have used some form of LCA to develop federal packaging mandates that require manufacturers to take back packaging discards or pay for their recycling. Germany requires companies that do not participate in its Green Dot program to take back their packaging and pay the cost of recycling it themselves, with no excep-

tions for foreign companies. This measure has broad implications since the take-back burden is far greater for those companies that ship their products long distances to Germany. Thus several manufacturers exporting to Germany from within the EU and beyond argue that, due to its nature, the Green Dot label program places imported goods at a market disadvantage. Moreover, industry and trade organizations within Europe argue that the degree of diversity between countries and even regions within the same country is so large that the preferred waste management method in one area may not be appropriate for other areas. Thus, these constituencies claim that waste management decisions should be made on a case-by-case basis (Europen, [123]).

There are no federal packaging mandates of a similar kind in the United States (U.S. EPA, [125]). However, there are a number of federal and state initiatives that involve the use of LCA based tools. For instance, since 1997, the U.S. EPA has been promoting the concept of extended product responsibility (EPR) which is a product-oriented instead of a facility-oriented approach to pollution prevention by using product life cycle concepts [126]. Within this principle, programs such as Environmentally Preferable Purchasing (EPP) promote the use of LCA-based tools. In fact, originating from executive Order 13101 on "Greening the Government through Waste Prevention, Recycling, and Federal Acquisition", EPP is a nationwide program that uses the leveraging strength of federal buying power as an incentive for industry to develop environmentally preferable products. Guidelines for EPP are developed by the U.S. EPA for use by other federal agencies; however, the program encourages state and local government and the private sector to incorporate environmental considerations into their purchasing processes as well.

Another kind of initiative is the U.S. EPA Design for the Environment Program (U.S. EPA-DfE) [125] which is a voluntary government-industry partnership that seeks to incorporate environmental considerations into the design and redesign of products, processes, and technical and management systems.

Outlook and Conclusions

The future of LCA for packaging, as for any other product category (e.g. energy, automobiles, appliances), is necessarily linked to the future of LCA and its maturation into a more reliable tool.

Thus, with regard to LCA in general, challenges remain due to the uneven pace at which it has been embraced around the world. In fact, though developed more than thirty years ago in the U.S., the European willingness to incorporate it as a part of their environmental regulatory process is often cited as the reason why Europe leads the way in LCA research [127]. On the other end, it is only since the 1990's with ISO's release of its 14000 series of standards on Environmental Management that many developing countries have started to learn about this concept. The delay in coordination (i.e. internationally and nationally) is one reason why a common terminology has been slow to develop, and terms and approaches such as life cycle management or life cycle costing generate confusion. Further, since many LCA studies still remain unpublished or inaccessible, the assimilation of common methodologies is even more difficult. On the other hand, the increasing tendency of the private sector to look at product life cycle concepts and embrace them at the product design phase in order to respond to consumer expectations may be a sign of what is next. As multinational firms extend their operations around the world, along are spread their philosophies and their understanding of LCA concepts. This is why efforts on harmonizing private life-cycle initiatives have started to occur. For example, under the UNEP/SETAC Life Cycle Initiative various workgroups on inventory, impact, and Life Cycle Management (LCM) are trying to achieve this international coordination and discussions have been proposed to adopt LCM as the platform from which to build and execute private environmental stewardship programs [128], with an "LCM toolbox" with LCA and LCC as components [70,129]. The open partnership of the private sector with environmental research institutes and regulatory bodies has often been cited to as one of the reasons why many European countries have a healthy LCA activity [130], and this is a reason why international harmonization measures in the private sector are important. Thus, coordination efforts reflected by the numerous guidelines from SETAC and ISO, and working groups and workshops will need to continue to catalyze the harmonization process and to address current limitations. Furthermore, private and governmental agencies of the U.S. and of European countries will need to continue their development of frameworks and partnerships with industry to help with the goal of making LCA a more useful tool for decision-makers. Current efforts by environmental certification third party organizations in these countries will need to focus on continuing their homologation steps as well as reaching

out to similar bodies in developing countries. Likewise, work remains to be done on controversial matters such as data quality and harmonization of inventory procedures and impact assessment methods as well. LCIA improvement will also depend on the success of efforts on modeling the fate of chemicals released into the environment and development of weighting procedures. But perhaps, due to the nature and subjectivity of many of the components of an LCA, several issues will still remain unresolved.

Challenges exist for LCA use for packaging. For example, as packaging remains a necessary item in the market and one of the preferred fields to which LCA is often applied, world population growth and a higher overall quality of living are indicators that packaging waste management options (e.g. reuse, incineration, recycling) will remain issues for further LCAs. Furthermore, optimization in packaging design with regard to the environment will necessarily use life cycle-based methods as expressed by emerging industry groups such as the Sustainable Packaging Council [131]. The consequences of trade agreements and a "global economy" with regard to production and movement of products and their packaging from one region to another and the consequent implications for resource (e.g. material and energy) use and emissions release will also have to be investigated with a "life cycle thinking" philosophy. Lastly, as ever more complex packaging concepts are designed in order to meet increasingly higher consumer expectations [132], complexities in their LCA will require further research. Improvements in radio frequency identification (RFID) technology for product tracking, new dynamic promotion/information capabilities through active labels, product quality sensors, nanoscale optimization of material properties, development of fully biodegradable as well as biodegradable composite materials are some examples of scientific breakthroughs that are starting to be used [133], Biodegradable Plastic Society, [134] or are being investigated to build packaging materials and packaging components [135,136] in the future. In turn, inventory databases will need to be developed not only for "conventional" packaging materials and components but for these new packaging concepts as well. Furthermore, since with the use of these new capabilities, new functions would be added to packaging (e.g. degradability, traceability, sensing) besides the traditional containment, the definition of the functional unit for an LCA will be a subject for future debate. All these challenges are indications that LCA research for packaging options should remain a high priority in the future.

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Material Innovation: Thermochromatic Inks

AUDREY MICHAEL WHALING

INTRODUCTION

TODAY, marketers constantly take new approaches with packaging to gain consumer recognition. Brand loyalty does not provide enough motivation anymore and now marketing has begun to use sensory stimuli to attract customers. Packaging manufacturers require in depth information of consumer psychology to understand consumer response to their packages (Siloyaoi 2004). The sight of a package and taste of a product had a strong hold on business in the past, but manufacturers have begun to use touch, smell, and sound to attract customers.

Many products tend to look alike on shelves and a consumer will make a decision to buy based on price rather than the appearance of the item itself (Taylor 2004). However, companies are gaining purchasing behaviors through the use of *thermochromatic inks.* A Thermochromatic ink is one that changes appearance during temperature changes. For example, should a consumer pick up a black can coated in thermochromatic ink, the heat from his or her hand would warm the can, increasing the temperature and resulting in a color change to red. Visual effects such as this tend to increase point of purchase activity, influence the consumer when purchasing, and will appeal to the consumer through visual differentiation (Aurenty 2004). On a shelf of 100 cans, the one that reflects or changes is the one that consumers will ultimately pick up. If a consumer handles a package, they will more than likely buy it.

One of the surest ways to get attention of consumers is to allow them to interact with your message (Daubert 2005). If a consumer can touch a product, ultimately making it change color, they connect with it on a higher level than if they were simply to see it. A typical package without any specialty ink connects to a consumer through only one aesthetic avenue; sight. A package that has thermochromatic ink can connect to a consumer through two different avenues; sight and touch.

What is a Thermochromatic Ink?

Thermo is prefix used when referring to heat or temperatures. Chromatic is a term that means "of or relating to color or color phenomena or sensations" (Merriam Webster Online 2005). Of course ink is any liquid or paste like material that may or may not be pigmented, intended for the use of printing or writing. Putting all of these definitions together a thermochromatic ink is one that is used for printing and has the ability to change color with varying temperatures.

Thermochromatic materials were first patented in 1982 and are characterized as a material that "undergoes reversible metachromatism at a temperature within the range of –50º C to 120º C. Furthermore, the reversible thermochromic material may be contained within microcapsules," (Kito, et al. 1983). They were invented at the Pilot Ink Company and today have found a unique niche in the ink market. These specialty inks have been used in paints, clothing, paper, and security seals. Only recently have they been applied to packaging, but the consumer interest and interaction with them has opened the door for manufacturers to take a better look at these sense-arousing products.

How it Works

Thermochromatic inks exist in two forms; as liquid crystal and as Leucodye thermochromatics. In liquid crystal inks, crystal structures exist at very low temperatures. At very high temperatures the material is in liquid form. In between these temperatures, the material is a "cholesteric liquid crystal" (White and LeBlanc 1999). In this region, as the substance heats up, a color change from black to red up through to blue and violet can be observed.

Bragg's law suggests the incoming light will hit the cholesteric liquid crystal and will reflect. For example, if a crystal is held to the sun, certain parts of the incoming light are reflected, giving off a shimmering,

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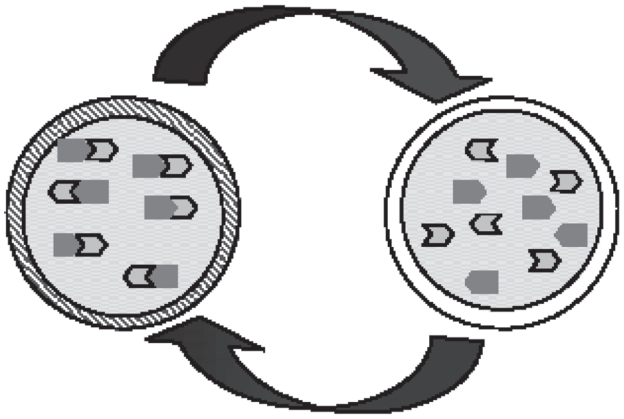

Figure 1. *Schematic of Leucodyes (Sun Chemical 2005).*

multi-colored sparkle. The same idea holds true with the liquid crystals. As the liquid crystal phase warms, it changes and breaks up. The incoming light will reflect differently off the multiple orientations of the crystals, giving off different colors. At lower temperatures, wavelengths around 600–700 nm are reflected showing colors of red and orange. As the temperature increases, wavelengths of 400–500 nm reflect and colors like blue and violet are shown (Glogowski 2003).

Applications of liquid crystal inks can be found in forehead thermometers, aquarium temperature records, the famous "mood ring," or in other areas where a highly temperature sensitive product is needed. However, because of their sensitivity to heat, liquid crystal inks are extremely hard to handle and print.

A Leucodye has two components; the dye and the color developer. The dyes are usually colorless or "white" unless in the presence of the developer. These are carefully mixed together in a medium that is crystal at lower temperatures. When heated this medium dissolves and because the components are no longer together, the dye becomes clear again or "disappears." After the temperature returns to normal, the medium or microcapsules reform, encasing the dye and developer, and once again showing color (Sun Chemical 2005).

For effect on applications these temperature sensitive microcapsules are mixed into ink. For example, a yellow die may be mixed with a red Leucodye forming orange ink that can be printed. When the temperature of the substrate material increases, the microcapsules dissolve, breaking apart the dye and developer. This makes the red dye "disappear" revealing the yellow. This technique can also be used to put a coat of ink over a picture (Chromatic Technologies 2005). When the ink warms, the picture appears and disappears again when the ink cools. Leucodye based thermochromatic inks find many applications in clothing, printing, and even packaging.

Table 1. Color Range at specific temperatures (Color Change Co. 200).

Color Range		Temperature Range	
Color	**Pantone**	**Centigrade**	**Fahrenheit**
Red	186C	–5°C	23°F
Rose Red	217C	1°C	34°F
Magenta	675C	8°C	46°F
Vermillion	1785C	23°C	73°F
Orange	172C	29°C	84°F
Yellow	393C	31°C	88°F
Yellow Green	359C	33°C	91°F
Charm Green	373C	35°C	95°F
Green	3435C	36°C	97°F
Sky Blue	2925C	37°C	99°F
Turkish Blue	320C	40°C	104°F
Blue	285C	43°C	109°F
Dark Blue	287C	45°C	113°F
Violet	286C	50°C	122°F
Black	Black 3C	60°C	140°F

Due to the difficulty of handling and processing of liquid crystal thermochromatics, this paper will focus primarily on leucodyes and their applications.

Typically leucodye based inks require a minor change in temperature; about 3°C (5°F) or more (Johansson 2004). A wide range of colors exists over a vast range of temperatures in thermochromatic inks. Temperatures starting at –25°C (–13°F) and rising to 65°C (149.0°F) can have a color change, whether it is one color to clear, or one color to revel the color printed underneath.

APPLICATION

Typically thermochromatic ink can be applied by any conventional method. "Depending on the application, color-changing inks can be applied with a number of printing processes, including offset lithography, flexography, gravure, and screen printing" (Homola 2003). However, due to the microencapsulated dies, the inks must be printed with very low impact or no impact at all (Latunski 2005). Impact printing may damage the encasings and destroy the effect of the ink.

Screen Printing

A mesh screen is used with a design or words cut out of it. It is placed over the substrate or material to be printed and a small amount of ink is added at the top end

Figure 2. *Screen printing (Dharma Trading Co. 2005).*

of the screen. A squeegee is pulled down pushing the ink along the screen, and where the cut outs exist, the ink permeates through onto the substrate. The screen can be cleaned and reused and the ink used until it is gone.

When this method is used, manufacturers must be careful in the size of screens they use. Because of the microcapsules, the holes in the screen must be larger than normal to allow them to permeate through onto the substrate. This requires the screen be loosely woven and around the 110-230 thread per inch range (Homola 2003). Additionally, this requires increased drying times for UV and epoxy inks. Although this is a non impact type of printing and will not damage the microcapsules, screen printing is slow and tends to have poor graphics.

Lithography

A lithographic printing process requires an image to be transferred onto a plate via photographic exposure, or exposure to an intensely bright light. After exposure the image area is treated with chemicals so that it may retain oil based inks. The rest of the plate is treated to repel the oil and accept water, thus lithography works because oil and water do not mix. When printing occurs, the image on the plate picks up the ink and transfers it onto a blanket that THEN transfers it to the material being printed (PNEAC Online 2005).

Due to the imprinting experienced when the ink is transferred from the rollers to the plate to the blanket and then to the substrate, most of the microcapsules are destroyed. Although they can be modified to be more durable, a printer runs the risk of losing the inks characteristics.

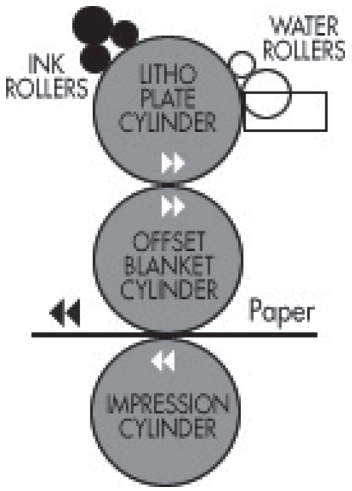

Figure 3. *Lithographic Printing (About.com 2005).*

Flexography Printing

In this method of printing a roller picks up ink and transfers it to an Anilox roll that evens it out as it rotates onto another cylinder containing the plate. This plate roller revolves against an impression cylinder through which the substrate passes. The pressure of the impression cylinder on the plate transfers the inked image onto the material being printed. This goes into drying before it is printed again. Typically at the end of this cycle, the roll of substrate is cut and rewound.

Once again, due to the impact from the transfer of ink to plate to substrate, the microcapsules can be destroyed. Although this impacting is not as great as it is in lithography, the ink can still be damaged so that it will not change color properly. Additionally, if the microcapsules are too large, they may get caught and build up behind the doctor blade used to wipe away excess ink.

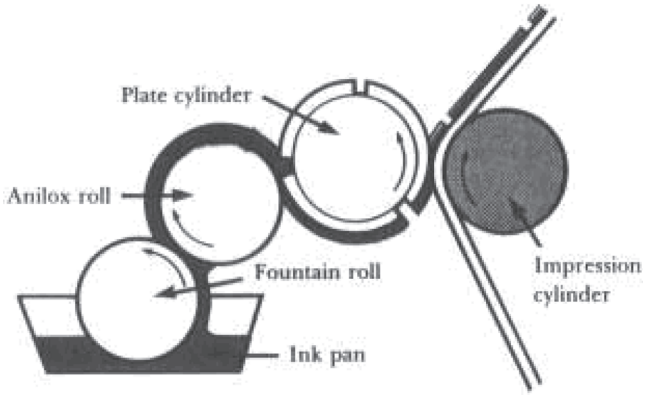

Figure 4. *Flexography Printing (PNEAC online 2005).*

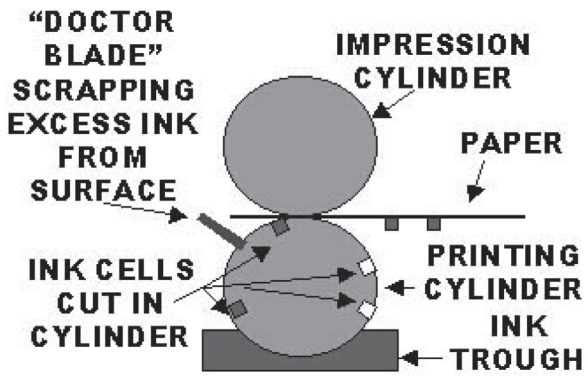

Figure 5. *Gravure Printing (Stamphelp.com 2005).*

Gravure

With Gravure printing, a plate is engraved or imprinted with a design of what needs to be printed. Ink exists in cells in a cylinder and rotates against the impression cylinder with the indentations on it. The substrate or paper travels in between the two rolls and the ink finds its way into the indentations of the impression cylinder. This leaves a print or "well of ink" on the paper.

Because this printing method needs ink that flows well, some restriction is placed on the size of the microcapsules. Ink must have a high flow (low viscosity) in order to properly coat the plate, therefore placing more limits on variety of inks used. Again, a doctor blade exists to wipe of excess ink on the surface. If the microcapsules are too large in size, they will get caught behind it and make printing difficult. However, this printing method has very little or extremely low impact, making it a wise choice for thermochromatic inks.

All of the above methods can be used to print thermochromatic inks onto various substrates. Each has its advantages and disadvantages and it depends on the product as to what technique to use. There is no real limit as to what substrate can be used so long as it is compatible with the ink. The thermochromatic ink will only be as good or last as long as regular ink would with that substrate. The inks themselves have a shelf life of about six months, but after printing they can last for several years and undergo thousands of color changes without losing any characteristics. However, if the ink is exposed to extreme heat, cold, ultraviolet rays, or any solvents that could damage the ink itself, the shelf life is dramatically reduced. Additionally if the substrate the ink is printed on gets damaged, it will affect the performance of the ink overall.

USES

In the past, thermochromatic inks have been used in mood rings, thermometers, Hypercolor clothing, and for security purposes with documents and other products. Today, these inks continue to find new applications and have become an interactive part of consumer products and in the food packaging industry. Creating an experience with the package forms "a unique relationship in which consumers and brands connect from an emotional an individual perspective…this experience results in positive purchasing decisions and strengthens brand loyalty (Glass 2004). Food product manufacturers can benefit from increased purchasing, thus profits. Although currently their use is relatively small, the world of packaging is quickly moving toward using thermochromatic inks to enhance consumer experience and buying of products.

Security Purposes

Some security papers are printed with thermochromatic ink in words saying "VOID" or "COPIED" that are invisible when the paper is serving its function (Rippedsheets.com 2005). When the paper is copied, the heat from the copier activates the ink, and the words appear to indicate the document has wrongfully been used. Others are coated with a layer of ink that disappears when heated. If a check, coupon, or document were copied, the special feature of the thermochromatic ink would not exist on the duplicate. The receiver of the item could quickly confirm if it were valid, simply by warming it and observing or not observing the expected color change. The same feature can be applied to security seals on documents; if the color changes with the heat of a hand, the document is genuine (Honig 2005).

Additionally thermochromatics could be used to authenticate expensive products. In 2002, *Wine Business Monthly* discussed trouble with counterfeiting of wine labels. Several instances of cheap wine marked with fake labels inspired the industry to take action against criminals that not only made profit off of their meddling, but tarnished the image of the wineries in doing so. Honig (Honig 2002) suggested using thermochromatic inks on labels to ensure the product is authentic: "Difficult to duplicate and easy for customers to detect, thermochromatic inks are becoming popular among printers of bank checks, and show great promise in the wine and packaged goods industries."

Security is not only needed with documents, but also with products as well.

Hypercolor Shirts

Hypercolor shirts were a fad of the late 80s and earlier 90s that took advantage of the characteristics of thermochromatic inks. The fabric of the shirt was dyed a particular and constant color. The shirt was then dyed or coated with leucodyes that formed a different color when added to that of the shirt. As the shirt warmed, the leucodye capsules dissolved and the color became clear, showing off the dyed fabric of the shirt. When the fabric cooled again, the shirt displayed the color combination of the fabric and leucodye.

Hypercolor shirts were extremely popular but experienced several problems. If the fabric were exposed to bleach it would damage the microcapsules and ink, destroying the color change affect of the shirt. Exposing the shirt to extreme heat (like that of a dryer) also diminished the color changing ability. Finally, after several washes, the color of the shirt began to fade in addition to the leucodye pigment, diminishing the quality of the product. Due to the vigorous handling, the idea of Hypercolor clothing was all the rage, but only the fad faded fast.

Packaging

Lately a struggle has ensued on how to capture the interest of a consumer. Companies are finding they can no longer rely on brand loyalty, quality, or competitive pricing to draw consumers. Manufacturers are quickly realizing that adding value to packaging has to come in ways that connect with the consumer through other emotional avenues. Package interaction has been a successful solution to this problem. And what better way to interact with a package/product system than through thermochromatic inks?

Using thermochromatic inks, consumers can detect when a product is ready for consumption. For example, putting a container of soup in the microwave, a consumer must continually check if it reaches the correct temperature. Furthermore, they run the risk of burning themselves on the product. If thermochromatic ink were printed like a thermometer on the outside of the container, as it heats up, it could communicate to the consumer when the ideal temperature is obtained. This saves the consumer time and grief, adding value overall. Hungry Jack® used this concept with its breakfast

Figure 6. *Breakfast Syrup (Color Change Corp.2002).*

syrup. A small panel on the front indicates the package is hot and ready for consumption (Homola 2003).

In the same sense, when a package reaches a temperature cool enough for consumption, a thermochromatic indicator could display that information. Rather than testing the juice to see if it is cold yet, a consumer has the option to glance at a label and find out.

Chromatic Technologies, Inc. used this idea on its Hite Beer. The ink changed color as it cooled indicating the product is ready for consumption. The Dutch company, *Toorank*, used a temperature sensitive label on the back of their Petrikov vodka. The label included a hidden message of "OK! I'm cool! Drink me now!" that appeared at 8°C (Packworld.com 2004).

With these inks, manufacturers also place curiosity and the idea of package novelty in the hands of the consumer. The boxed set of CDs of Lights Out by Nirvana incorporated the use of thermochromatic inks. On a part of the package, thermochromatic inks were placed over printing to "hide" what was underneath. When the ink was warmed, it disappeared to show information about the band (Maselli 2004). Another product from Fort Dearborn Company, Glade® Magic Candles, had the

Figure 7. *Beer Can (Packworld.com 2004).*

candle sleeve printed with thermochromatic ink. Upon heating of the candle, the printed design vanished and a butterfly boarder appeared (PLGA Online 2004).

Manufactures have the ability to capture children's attention as well. Chromatic Technologies, Inc. developed several interactive packages including a Pop Tarts® box with a question and answer game on the back; a PlayStation2® video game cover that changes colors when handled; and a Pringles® can that reveals a hidden message (Chromatic Technologies Inc. 2005).

CONCLUSIONS

Customers who are more "in touch" with a package are likely to buy it and display brand loyalty in the future. The curiosity, emotional connection, and interest in a product/package will keep them coming back for more. Thermochromatic inks are an excellent way to achieve package interaction as they fulfill the need for utility, communication, and motivation. Mr. Bob O'Boyle, director of USA technology transfer at Sun Chemical suggests, "Interactive packaging, to me, seems the largest area for growth…Labels or containers sensitive to heat at various levels could be used for safety (too hot), on beverages (cold) to anything else a producer would like to convey to a consumer" (Agosta 2002). These added benefits are added value; something that consumers constantly look for. Packaging manufacturers would be wise to get on board and give the consumer a whole new dimension of packaging.

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Table 5. Comparison of state-of-the-art matrix resins with VPSP/BMI copolymers.

Resin System	Core Temp. (DSC peak)	T_E	Char Yield, %
Epoxy (MY720)	235	250	30
Bismaleimide (H795)	282	>400	48
VPSP/Bismaleimide copolymer			
C379: H795 = 1.9	245	>400	50
C379: H795 = 1.4	285	>400	53

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