

**Potential Migration of ϵ -Caprolactam to Water
and Wine as Affected by Transportation and
Storage**

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Master of Science in Agriculture with specialization in Crop Science

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Committee Membership

TITLE: The Potential Migration of E-Caprolactam to Water and Wine as Affected by
Transportation and Storage

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Abstract

Under normal sealing and storage conditions, Nylon-6, poly-caprolactam-based plastic laminates may release impurities to packaged foods and liquids and the application of heat for cooking often increases the rate of migration. Epsilon-caprolactam is one of the main contaminants found to migrate from a Nylon-6 poly-caprolactam plastic film. The objectives of this study were to determine the effects of solvent, transportation and storage time on the migration of ϵ -caprolactam from a Nylon-6-based lidding material into water or a white wine substitute (12% ethanol). Polypropylene plastic cups were filled, sealed, packaged, stacked and exposed to a simulated 3-day cross-country shipment. Cups were sampled with or without simulated shipment after 0, 7, 14 and 28 days at 20.6°C. Epsilon-caprolactam was determined using a GC equipped with FID and a Restek Rtx 1301 megabore column. Results of the study indicated migration of ϵ -caprolactam into containers at the time of sealing with significantly higher levels (4.42 ppm average) occurring in cups containing 12% ethanol vs. water (0.01 ppm average). After the cups were sealed, neither simulated cross-country shipment nor storage increased levels of ϵ -caprolactam in either solvent. The results indicate that wine sealed in packages lined with Nylon-6-based plastic could contain significant amounts of ϵ -caprolactam. However, it is not understood how the alcohol, whether as liquid or vapor, interacted with the lidding material to increase migration at the time of sealing. No delamination of the polypropylene layer in the lidding material was observed after sealing. Future research needs to be conducted to study the effects of alcohol, alcohol vapor, sealing time and temperature on ϵ -caprolactam migration.

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Literature Review

Nylon 6 is a polyamide commonly used in the production of multi-layer plastics used for packaging and the storage of foods and liquids. Nylon-6 plastics are also used to manufacture the casings for sausages or cooked meats and as bags (monolayer or laminate) for boiling, microwaving or roasting foods (Stoffers et al., 2005). Nylon 6 is produced by the hydrolytic polymerization of ϵ -caprolactam (Soto-Valdez et al., 1997). Epsilon-Caprolactam is a cyclic amide used in the production of synthetic fibers such as Nylon and Perlon (Pogorzelska et al., 2001) and is synthesized from cyclohexanoxime using an acid-catalyzed rearrangement of the oxime to an amide (polyamide 6) (Fig. 1). This process is commonly referred to as the Beckmann rearrangement reaction (Marziano et al., 2008).

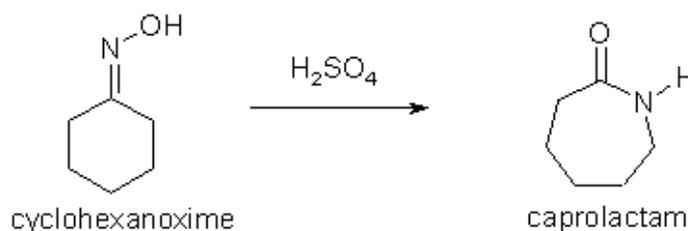


Fig 1. Synthesis of caprolactam from cyclohexanoxime (from Marziano et al., 2008).

Synthesis of Nylon 6 begins with the carbonyl oxygen of the Caprolactam molecule passing a pair of electrons to the hydrogen molecule of water and the hydrogen atom attaching to the carbonyl oxygen forming a protonated carbonyl group and a free hydroxyl group (Fig. 2A). The free hydroxyl group then combines with the positively

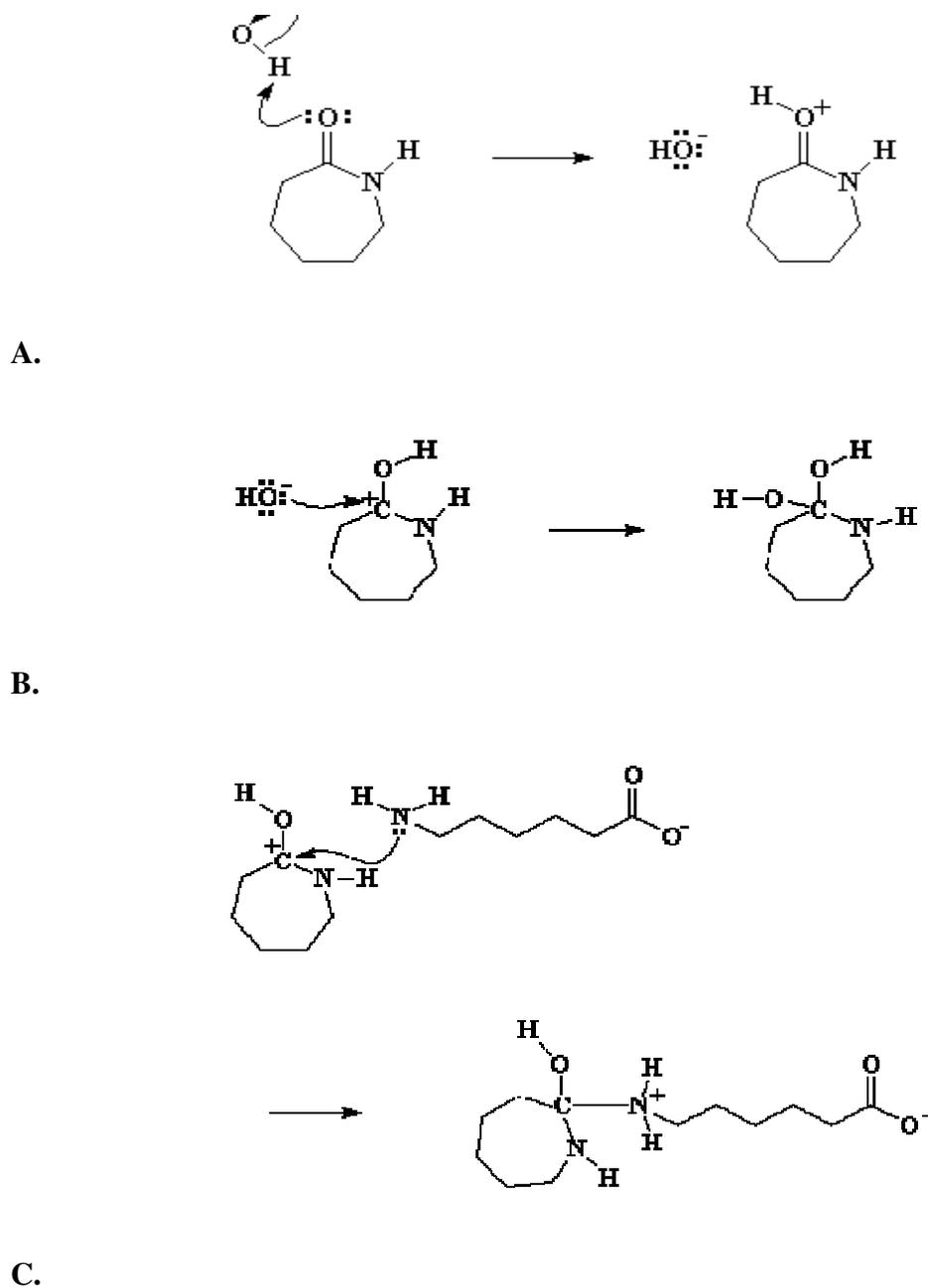


Fig. 2. Synthesis of Nylon 6 from ϵ -caprolactam. A) Caprolactam molecule uses a hydrogen molecule to create a protonated carbonyl group (carbocation). B) Hydroxide ion combines with the carbonyl group to create an unstable gem diol. C) Linear amino acid combines with carbonyl group creating an ammonium species (from Anonymous, 2005a).

charged carbonyl carbon atom forming an unstable gem diol (two hydroxyl groups attached to the same carbon atom). Since the gem diol is unstable, electrons rearrange breaking the amide ring and forming a linear amino acid, which reacts with other caprolactam molecules creating another carbonyl molecule (Fig. 2B). However, instead of this carbonyl group reacting with the free hydroxyl group from the water molecule, the molecule reacts with the amino acid creating an ammonium species. After proton rearrangement, another linear molecule is formed (Fig. 2C, D). The carboxylate group at the end of the linear molecule takes the alcohol hydrogen regenerating carboxylic acid and forming a carbonyl group in the middle of the linear molecule (Fig. 3A). The acid reacts with other caprolactam molecules creating long chains of Nylon 6 (Fig. 3B) (Anonymous, 2005a).

The hydrolytic polymerization of ϵ -caprolactam to polyamide-6 is only 90% efficient (Barkby et al., 1993). Therefore, residues are left in the plastic, consisting of mixed monomer and oligomers that have the potential to migrate into food or liquid in contact with the plastic (Bradley et al., 2004). Some of these residues can be eliminated from plastic surfaces by leaching or vacuum treatment; however, leaching only removes another 9%, leaving 1% residual on the polymer. Barkby et al. (1993) reported residual extractable levels of Nylon 6 oligomers around 1-4% w/w.

The migration of contaminants to food is not limited to caprolactam. When analyzing roasting bags, Soto-Valdez et al. (1997) identified 13 migrants from the nylon microwave and roasting bags including ethyl acetate, benzophenone and tetradecane. Each of these migrants can have significant health effects if they are ingested, inhaled or come into contact with skin (Anonymous, 2007a).

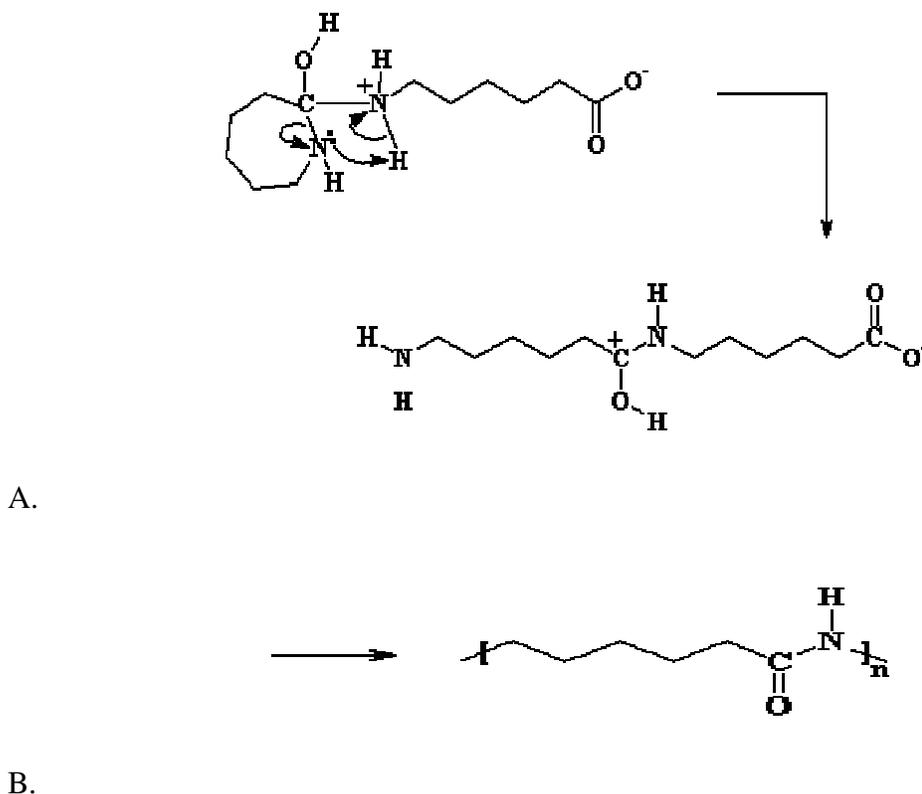


Fig. 3. Synthesis of Nylon 6 from ϵ -caprolactam. A) Through electron rearrangement, carbocation ring opens creating a linear molecule. B) The dimer then reacts with more caprolactam molecules forming long chains of Nylon-6. (From Anonymous, 2005a)

Epsilon-caprolactam is a white, hygroscopic, crystalline solid that is extremely soluble in water (Anonymous, 2007a). Although the EPA has classified ϵ -caprolactam as non-carcinogenic, acute inhalation exposure to ϵ -caprolactam has been shown to cause irritation and burning of the eyes, nose, throat and skin, and headaches, malaise, confusion and nervous irritation in workers (Anonymous, 2007a). Neurological, gastrointestinal and cardiovascular effects as well as dermatological and immunological changes were reported in workers that were chronically exposed to caprolactam.

Studies/Research

The National Academies Board on Environmental Studies and Toxicology (BEST) reported that ϵ -caprolactam is rapidly and completely absorbed in the stomachs of mice (Ramanathan, 2007). However, much of the research reported by BEST involved toxicity testing for ϵ -caprolactam at concentrations in excess of 600 mg/kg of body weight. These concentrations are extremely high and have so far not been found in food or beverages packaged or stored in ϵ -caprolactam-based polymers (Ramanathan, 2007).

NASA conducted a study to determine the potential for migration of ϵ -caprolactam into water (Ramanathan, 2007). Aboard the Space Shuttle, potable water was stored in contingency water containers (CWCs). CWC's are multi-layer plastic bags lined with a material called Combitherm, which contains Nylon-6 (Anonymous, 2008a). A taste test was conducted with water stored in the bags for 12, 48 and 64 weeks (Ramanathan, 2007). The water was of the same quality as the water normally given to astronauts on the space shuttle or the International Space Station (ISS) and contained minerals, and silver biocides as disinfectants. The concentration of ϵ -caprolactam in the water was 4.7, 11.5, and 12.6 mg/L, respectively. ISS water was also compared with tap water from the Johnson Space Center and retail bottled water. At the maximum level of 12.6 mg/L of ϵ -caprolactam, the study concluded that the water was acceptable for drinking purposes as no off-flavors were reported. It was noted that test subjects sampled the water only once and were not asked to evaluate the water for an extended period (Ramanathan, 2007).

The United States has legislation requiring that all plastics in contact with food not significantly migrate into the food (Lawson et al., 1995). Title 21 Section 177.1500 of the Code of Federal Regulations (U.S. Gov't Printing Office, 1977) establishes the testing

requirements and migration limitations of the component materials in Nylon resins.

These limitations are based on the minimum extractable fraction in four solvents: water, 95% ethyl alcohol, 100% ethyl acetate and 100% benzene. Concentration limits for each solvent are 1.0%, 2.0%, 1.0% and 1.0%, respectively (U.S. Gov't Printing Office, 1977).

European Union regulations establish a specific migration limit (SML) for ϵ -caprolactam of 15 mg/kg of foodstuff (Anonymous, 1982). The SML's are based on time and temperature and have been specified for four solvents: distilled water, 3% acetic acid, 10% ethanol and rectified olive oil. Migration limits have been established to ensure the protection of consumers; however, research has indicated problems with the SML's established for plastic materials. Philo et al. (1994) reported that some monomers, such as propylene oxide, styrene and ethylene glycol, can experience up to 100% decomposition when exposed to the conditions of the EU testing protocol. Migration testing protocols and migrant limits in foodstuffs (SML's) assume monomer stability during exposure to heat and specific solvents.

Migration studies have tried to simulate retail and consumer situations involving microwaveable meals, sausage casings or roasting bags. It has been common to test bags or pouches for plastics migration directly into foodstuffs or the aqueous solutions surrounding the foods. It was found that heat caused the migration of plastic components into both the food and surrounding solution (Gramshaw et al., 1998; Lawson et al., 1996; Sarbach et al., 1996).

Studies have usually determined contaminant levels by cutting and boiling small squares of plastic in water or oil, then analyzing the solvents (Barkby et al., 1993; Soto-Valdez et al., 1997). Barkby et al. (1993) analyzed ϵ -caprolactam and residual cyclic

oligomer migration from Nylon 6 packaging into boiling water. Two films, 15 μm and 80 μm in thickness, were cut into 1 cm squares and dropped into boiling water. Both films released ϵ -caprolactam into the water solution but the thicker film ultimately released more ϵ -caprolactam though the complete extraction of cyclic oligomers took more time. A loss of 1.0% w/w of the plastic was reported for both films in the initial hour of boiling and more weight loss occurred over the next 4 hours. It was found that thickness, type of film (blown or cast) and extraction time affected the amount of migrants found in the solvent. It was hypothesized that contaminants such as ϵ -caprolactam would migrate more into the water used to cook “boil in bag” food than into the food itself. Most commercially-produced products have some type of polyolefin and adhesive barrier next to the food, and ϵ -caprolactam is very water-soluble and therefore prone to migrate into water rather than oil or fatty foods.

Lawson et al. (1996) tested plastic laminates, including polyolefins (LLDPE), polyamides (Nylon-6) and polyurethane adhesives for their potential to contaminate “heat and eat meals” and “boil in bag” foods (Fig. 4). Migration of contaminants from multi-layer polyethylene (PE)-nylon bags was determined by exposing the pouches filled with olive oil to boiling water and sampling at 1 hour intervals. It was found that ϵ -caprolactam migrated across the adhesive and PE layers of the bags resulting in concentrations much higher in the cooking water than in the oil. This would be important if the water were later used to prepare other foods.

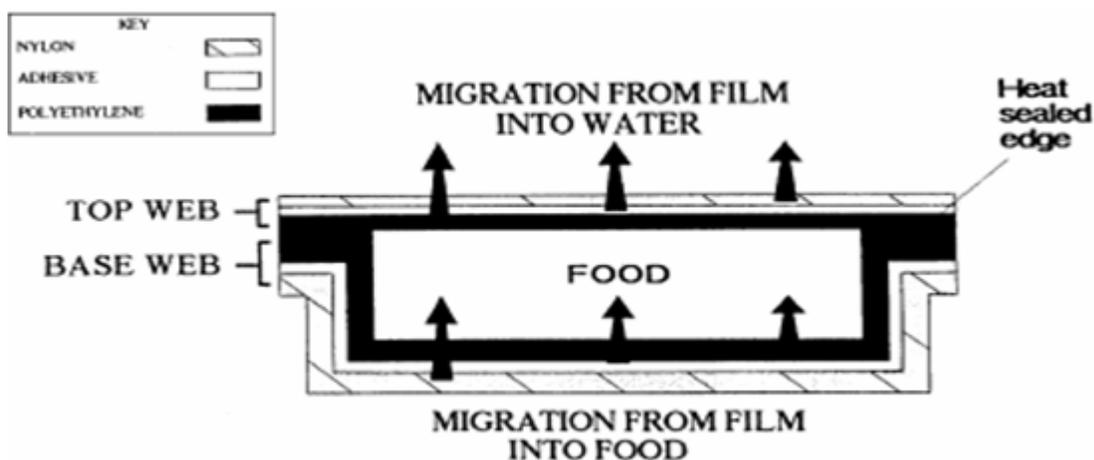


Fig. 4. Possible routes for migration from a boil in bag package during reheating food in boiling water (Lawson et al., 1996).

Soto-Valdez et al. (1997) evaluated nylon-based roasting bags to determine the migration of ϵ -caprolactam when exposed to high temperatures. Retail multi-layer roasting bags were used to make 10 x 15 cm pouches which were then filled with olive oil. The pouches were heated for 1 hour at 175°C, allowed to cool and the oil was analyzed. Epsilon-caprolactam was one of the main migrants found in the oil. Other materials such as cyclopentanone and cyclic compounds of Nylon-6 and Nylon-6,6 were also present. Nearly 42% of the Nylon 6 and Nylon 6,6 cyclic monomers and oligomers present in the roasting bags before testing migrated into the olive oil after heating.

Due to the hygroscopic nature of ϵ -caprolactam, the water concentration in Nylon-6 plastics can vary depending on the relative humidity of the environment (Soto-Valdez et al., 1997). Soto-Valdez et al. (1997) captured the water vapor emitted from a Nylon 6 plastic during heating (Fig. 5). Nearly 2 ml of condensed water vapor was collected in

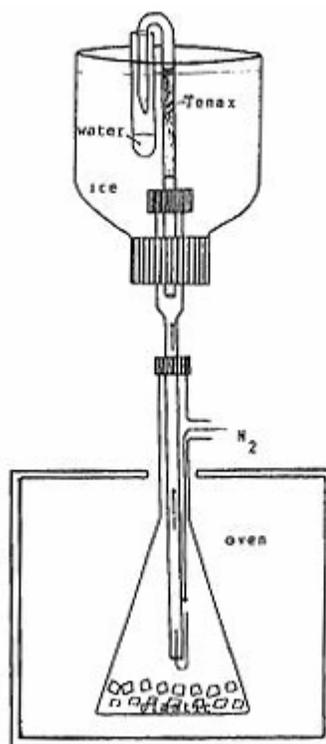


Fig. 5. System for trapping volatile compounds from multi-layer roasting bag material (Soto-Valdez et al., 1997).

the trap and analysis showed the presence of ϵ -caprolactam as well as other water soluble volatile compounds such as cyclopentanone.

Epsilon-caprolactam was found to be the main migrant from a tri-laminate bag consisting of polypropylene (PP), Nylon-6 (PA) and polyethylene (PE), and containing a parenteral (intravenous (IV)) solution of 0.5% metronidazole (Sarbach et al., 1996). In addition to the ϵ -caprolactam, other compounds were also detected in the IV solution including methylene dianiline (MDA) and several non-identified compounds thought to be plasticizers used in the manufacture of the bags. The presence of the ϵ -caprolactam confirmed its potential to migrate from the PA layer across the PE layer and into the IV solution.

Bradley et al. (2004) sampled numerous retail “boil in bag” and microwave roasting bags to determine the migration of Nylon-6 monomers. The three main types of packaging studied were: pouches intended for cooking by boiling in water, microwaving or roasting; the casings of cooked products; and packages for chilled foods where the food will not be reheated. Liquid chromatography-mass spectrometry (LC/MS) determined that when foods were microwaved in their packaging, ϵ -caprolactam migrated from the Nylon 6 film at levels ranging from 3.6 - 12 mg/kg foodstuffs. When comparing plastic samples that could be either microwaved *or* boiled in water, ϵ -caprolactam concentrations were much higher in the microwaved samples and ranged from 4.5 - 11 mg/kg compared to 0.15 – 0.93 mg/kg foodstuff for the “boil in bag” treatment. The lower results for the “boil in bag” samples were attributed to the migration of ϵ -caprolactam into the water rather than the food, though Bradley et al. (2004) did not test the water for ϵ -caprolactam concentration. Of the samples tested that were not re-heated after purchase by the consumer, ϵ -caprolactam levels ranged from 2.8 – 13 mg/kg foodstuff. However, all samples tested show levels below the maximum SML of 15 mg/kg.

Pogorzleska et al. (2001) tested plastic materials using European Union migration testing procedures, with the addition of 95% v/v ethanol as an alternate food simulant. Migration of ϵ -caprolactam from Nylon-6 film into a 15% ethanol solution ranged from 6.3 - 16.4 mg/kg solvent, exceeding the maximum SML for ϵ -caprolactam of 15 mg/kg foodstuff. Nylon-6 (PA) granules, Nylon-6 film, PA/PE laminate and PA mono- and multilayer casings were also tested in the study. When exposed to 15% ethanol, the PA granules released significant levels of ϵ -caprolactam and values ranged from 8.1– 46.5

mg/kg solvent. In 95% ethanol, ϵ -caprolactam concentrations were 7.1 – 42.7 mg/kg while in water, levels ranged from 7.4 – 56.6 mg/kg. All samples contained levels that exceeded the maximum SML's for ϵ -caprolactam. The migration of ϵ -caprolactam to olive oil occurred only with the PA film but levels were well below that of the other food simulants (1.5 – 3.8 mg/kg of solvent).

Gramshaw et al. (1998) measured the migration of ϵ -caprolactam and other volatile and non-volatile compounds into the meat, juice and skin of roasted chicken. The chicken was cooked at 200°C for 2 hours in a Nylon-6-based roasting bag. Using high performance liquid chromatography, it was determined that the concentration of ϵ -caprolactam was highest in the juices and skin. Epsilon-caprolactam levels were correlated with the fat content of each chicken part and direct contact of the plastic with the chicken increased migration. Concentrations of ϵ -caprolactam in the juices, skin and meat were 2.5 – 4.0 ug/g, 2.5 – 6.7 ug/g and 1.3 ug/g, respectively. Sixteen percent of the non-volatile compounds, including ϵ -caprolactam, present in the plastic roasting bag migrated to the chicken during roasting; however, only a small amount (1.44 mg/kg) of ϵ -caprolactam migrated.

Mathematical models can estimate the potential migration of contaminants to food simulants. Stoffers et al. (2005) used a migration cell to study the movement of contaminants to water or olive oil. This data was used to develop a mathematical model for a simultaneous two-sided migration test (Fig. 6) to estimate how plastics might react when placed between two liquids. Nylon 6 and Nylon 12 films, as well as one multi-layer film consisting of Nylon 6/Nylon 12, were tested to determine migration of

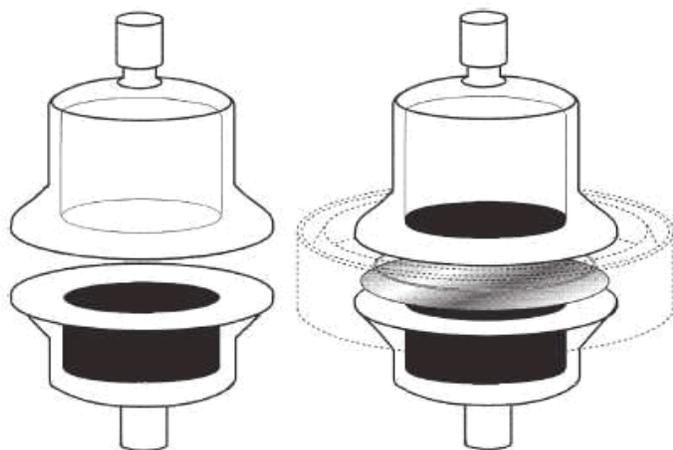


Fig. 6. Cell used for two-sided migration study (Stoffers et al., 2005)

contaminants. Incubation temperature was 100° C for 2 hours. The lower section of a test cell was filled with water and the upper section with olive oil (Fig. 6). Nearly 99% of the ϵ -caprolactam in the two Nylon-6 films migrated into water and with the multi-layer film, the ϵ -caprolactam migrated across the Nylon 12 (lauro lactam) layer into the water. Migration models indicated that the ϵ -caprolactam would migrate into the oil first, and by the end of the two hours, migrate back across that Nylon-6 layer to the water.

Bradley et al. (2004) reported findings on ϵ -caprolactam migration to foods that had only been exposed to heat during the package sealing process. Only one of the food products tested contained ϵ -caprolactam at a concentration above the limits of detection and the quantity, 0.12 mg/kg foodstuff, was well below the EU SML of 15 mg/kg foodstuff for ϵ -caprolactam.

Conclusions

Nylon 6 is one of the most commonly used polyamides in packaging (Stoffers et al., 2005) and retail applications include foodstuffs that, while packaged, are boiled, microwaved or roasted. High performance liquid chromatography, GC/MS, and LC/MS and have been used to test the migration of contaminants from plastic materials including Nylon 6. All have identified ϵ -caprolactam as a principal migrant when Nylon 6 plastic materials have been used to seal, store or cook commodities (Bradley et al., 2004). Due to high cooking temperatures, monomers of Nylon and other constituents of the plastic packaging have been found to migrate to water or the packaged food, and the duration of heating has been positively correlated with ϵ -caprolactam migration (Gramshaw et al., 1998; Lawson et al., 1996; Sarbach et al., 1996). Results also indicate that solvent type and heating method can affect ϵ -caprolactam migration. Microwaving or roasting was found to cause more migration of ϵ -caprolactam to food compared to boiling [reference]. This was attributed to the presence of water surrounding "boil-in-bags" and the hydrophilic nature of ϵ -caprolactam. When foodstuffs were sealed in Nylon 6-based packages but not subsequently cooked in-package, ϵ -caprolactam levels in the foodstuffs was very low and well below the EU SML for ϵ -caprolactam (Bradley et al., 2004).

Ramanathan (2007) reported ϵ -caprolactam migration to Nylon-6-based plastic bags used for long-term water storage and Sarbach et al. (1996) reported ϵ -caprolactam migration to IV solutions from plastic containers. The bags used in both studies contained Nylon 6 and storage was for nearly two years at ambient temperatures (Sarbach et al., 1996).

Gramshaw et al. (1998) reported evidence that the different parts of a chicken cooked in a plastic roasting bag will contain varying concentrations of ϵ -caprolactam. Concentrations were correlated with the fat content of the chicken part and of the chicken with the surrounding plastic bag.

Research therefore indicates that ϵ -caprolactam migrates from packaging to food and that this migration is accelerated by both cooking and cooking method. However, current evidence suggests that in many situations, the levels of migrants in foodstuffs are acceptable as defined by both U.S. and European standards. Interestingly, in contrast to cooked foods, evidence indicates that wine or spirits packaged in containers lined with a Nylon-6-based plastic might dissolve appreciable and unacceptably-high levels of ϵ -caprolactam.

**Potential Migration of ϵ -Caprolactam to Water and Wine as Affected by
Transportation and Storage**

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Key words: nylon-6, lidding plastic, food safety, ethanol

Introduction

Nylon 6 is a polyamide commonly used in the production of multi-layer plastics used for packaging and the storage of foods and liquids. Nylon-6 plastics are also used to manufacture the casings for sausages or cooked meats and as bags (monolayer or laminate) for boiling, microwaving or roasting foods (Stoffers et al., 2005). Nylon 6 is produced by the hydrolytic polymerization of ϵ -caprolactam (Soto Valdez et. al. 1997). Epsilon-caprolactam is a cyclic amide used in the production of synthetic fibers such as Nylon and Perlon (Pogorzelska et al., 2001) and is synthesized from cyclohexanoxime using an acid-catalyzed rearrangement of the oxime to an amide. This process is commonly referred to as the Beckmann rearrangement reaction (Marziano et al., 2008).

Epsilon-caprolactam is a white, hygroscopic, crystalline solid that is extremely soluble in water (Anonymous, 2007a). Although the EPA has classified ϵ -caprolactam as non-carcinogenic, acute inhalation exposure to ϵ -caprolactam has been shown to cause irritation and burning of the eyes, nose, throat and skin, and headaches, malaise, confusion and nervous irritation in workers (Anonymous, 2007a). Neurological, gastrointestinal and cardiovascular effects as well as dermatological and immunological changes were reported in workers that were chronically exposed to caprolactam.

The United States has legislation requiring that all plastics in contact with food not significantly migrate into the food (Lawson et al., 1995). Title 21 Section 177.1500 of the Code of Federal Regulations (U.S. Govt. Printing Office, 1977) establishes the testing requirements and migration limitations of the component materials in Nylon resins. These limitations are based on the minimum extractable fraction in four solvents: water, 95% ethyl alcohol, 100% ethyl acetate and 100% benzene. Concentration limits for each solvent are 1.0%, 2.0%, 1.0% and 1.0%, respectively (U.S. Govt. Printing Office, 1977).

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Migration testing protocols and migrant limits in foodstuffs (SML's) assume monomer stability during exposure to heat and specific solvents.

Nylon-6 has been commonly used in tri-laminate lidding materials where the nylon comprises the middle layer of the film. During sealing, the lidding material is exposed to temperatures between 175 and 200°C and there has been some concern that the film can decompose during sealing releasing ϵ -caprolactam into foodstuffs (Philo et al., 1994).

In 2007, reports of off-flavors in a white wine-based/gelatin mixture were described in confidential settlement documents made available to the authors. Plastic cups filled with the mixture were sealed with a Nylon-6-based lidding material. Consumers reported “off-flavors” from some of the cups and subsequent testing revealed the presence of ϵ -caprolactam in the wine/gelatin mixture. The purpose of the current study was to determine the migration potential of ϵ -caprolactam from a Nylon-6-based, multilayer lidding film, as affected by solvent – water or 12% ethanol (a white wine simulant), cross-country shipment and subsequent storage.

Materials And Methods

A multilayer plastic lidding film was obtained from Momar Industries (Reno, NV). It consisted of a 0.012 mm polyethylene terephthalate (PET) top-layer followed by an ink layer, adhesive, 0.015 mm nylon layer and 0.04 mm polypropylene (PP) peel-seal inner layer.

Water or 12% ethanol was sealed in 118 ml PP soufflé cups using this film. The water was HPLC grade or Nano-pure filtered water. The white wine simulant consisted of 99.5% ethanol diluted to a concentration of 12% with HPLC grade or Nano-pure filtered water. Each cup was filled with 90 ml of solvent leaving approximately 1.5 cm of headspace between the liquid and the plastic material. The lidding material was sealed to the soufflé cup with a Haug (Morgan Hill, CA) heat sealer maintained at 190.6°C. Sealing time was 1 second. Under these conditions, there was little or no obvious delamination of the lidding material. Seal integrity was measured using a Series 2000 Integrity Tester (Test-a-Pack Systems, Orchard Park, NY) set to an altitude of 3048 meters and 10 sec hold time.

Vibration Performance Testing

Eleven regular slotted containers (RSC), constructed using C-flute corrugated fiberboard, were filled with 60 soufflé cups containing either water or 12% ethanol. Each RSC contained 3 layers of 20 cups each separated by corrugated sheet cutouts (Fig. 7). Layer configuration was 4 x 5 cups. The RSC's were 29.2 cm x 40.0 cm x 16.5 cm to prevent vertical or horizontal movement of the cups within the boxes after packaging. Each RSC was closed and sealed with 5.08 cm pressure-sensitive tape.

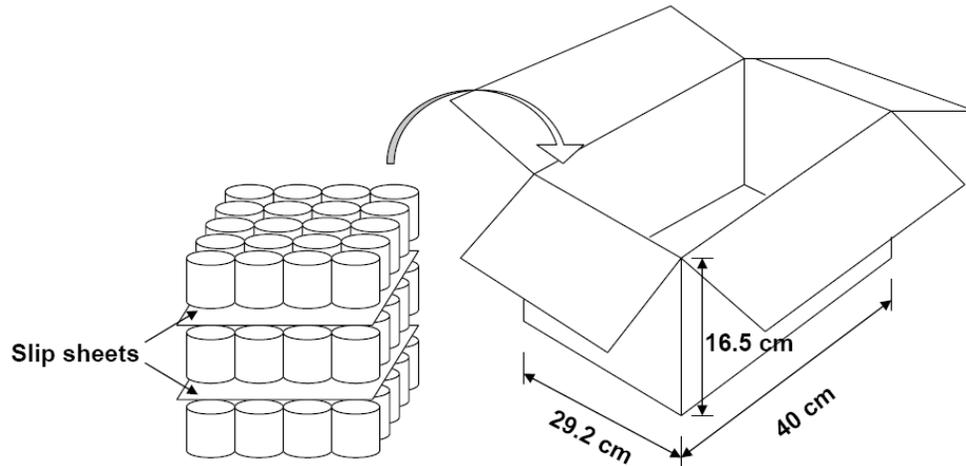


Fig. 7. Configuration used for transportation testing

ASTM standard D4169 (Standard Practice for Performance Testing of Shipping Containers and Systems) was employed to provide a uniform basis for evaluating the ability of the shipping units to withstand the vibrational forces encountered during distribution (Anonymous, 2005b). Schedule D from Standard D4169 indicated the protocol for random vibration and sinusoidal vibration tests.

The random test option (Anonymous, 2006b) for this study uses power spectral densities (PSD) to simulate truck-based distribution at Assurance Level II (Table 1; Fig. 8). The PSD is an expression of random vibration in terms of mean-square acceleration per unit frequency and the units are g^2/Hz ($g^2/cycles/s$). Power spectral density is the limit of the mean square amplitude (g^2 's) in a given rectangular band divided by the bandwidth, as the bandwidth approaches zero. Assurance Level II relates to mid-level test intensity and reflects the most likely probability of occurrence.

Frequency, Hz	Power Spectral Density Level, g^2/Hz
1	0.00005
4	0.01
16	0.01
40	0.001
80	0.001
200	0.00001
Overall, g_{rms}	0.52
Duration, min	180

Table 1: Power spectral densities for vertical truck Assurance Level II vibration.

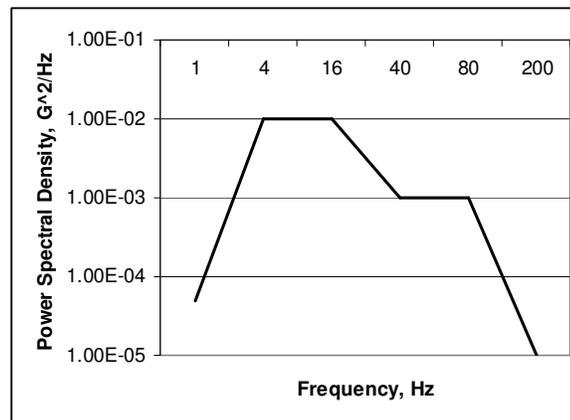


Fig. 8. Power spectral densities for vertical truck assurance Level II vibration.

The sinusoidal vibration test is also known as the “sweep and dwell” vibration test. Package systems are secured on the vibration table and a frequency “sweep” from 3-100 Hz is performed to identify the resonant frequency where the maximum vertical displacement of the packages is observed. The sinusoidal frequency identified through this test is then “dwelled” for 15 minutes (Anonymous, 2007b). The peak amplitude (0-peak) for this test is held steady at 0.5 g throughout the test.

Though the random test method provides a simulation of the actual transport vibrational environment, the sinusoidal test is commonly used in conjunction with the

random test as a means of determining and observing system resonances. The combination of the two tests is used to determine the ability of shipping units to withstand the vertical vibrational environment during transport and the dynamic compression forces resulting from vehicle stacking.

The RSC's for the current study were stacked vertically on a Model 1800 vibration tester (Lansmont Corp., Monterey, CA) and numbered 1 to 11; box 1 was at the bottom of the stack, box 11 was at the top (Fig. 9). The stack was surrounded by vertical aluminum restraints to restrict lateral movement. The top of the stack was not restrained so that vertical motion of the stack was not inhibited. The vibration testing was conducted under ambient conditions of 22.8°C and 50% relative humidity according to ASTM D4332 (Anonymous, 2006a).

Through a sweep and dwell resonance test, the resonance frequency of the stack was determined to be 3.8 Hz. The stack was maintained at this frequency for 15 minutes after which it underwent a 3-hour random vibration test to simulate the actual transport vibrational environment. Twenty randomly chosen cups were then taken from each sampling RSC (boxes 1, 6 or 11).

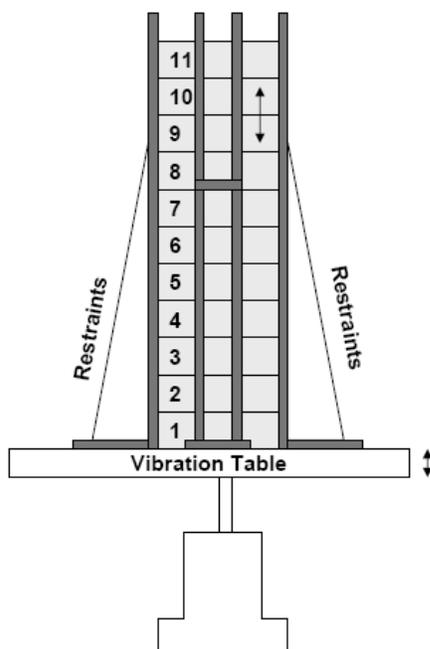


Fig. 9: Diagram of carton arrangement on vibration table.

Sampling Protocol

Sealed cups were either immediately stored at or subjected to a simulated 3-day cross-country shipment after which they were stored. Based on measurement of the ambient temperature in local retail supermarkets, shipped or unshipped sealed soufflé cups were stored at $20.6 \pm 0.25^{\circ}\text{C}$ (69°F). Cups were sampled at 0, 7, 14 and 28 days storage. On Day 0, samples were taken from the cups no more than 5 minutes after sealing (non-transportation study only). Samples taken on Day 0 from the transportation study were taken no later than 15 minutes after completion of the test. A sample was taken from each cup by breaking the seal and extracting the liquid directly with a glass micro-pipette.

GC Analysis

To determine ϵ -caprolactam concentration, 1 microliter samples were injected into a Shimadzu 2014 gas chromatograph equipped with FID and fitted with a Restek Rtx 1301 capillary column (15M, 0.53 mm ID, 0.50 μ m DF). Water samples were analyzed using a ramping function starting at 100°C for 1 min thereafter increasing 10°C/min to 240°C. The oven was held at 240°C for 35 min. Alcohol samples were analyzed using a ramping function starting at 60°C for 1 min thereafter increasing 10°C/min to a temperature of 240°C. The oven was also held at 240°C for 35 min. Helium was the carrier gas, the flow rate was 5 ml/min and the injection port temperature for all analyses was 225°C. An external standard of 5 ppm ϵ -caprolactam was injected at the beginning and end of every 15 sample injections. To eliminate buildup of contaminants on the GC column, 1 μ l of HPLC-grade acetone was injected between each set of 15 sample runs.

Experimental Design and Analysis

Treatments were completed randomized and repeated in triplicate. Data was analyzed using Statistical Analysis Software (SAS Institute, Raleigh, NC) and the PROC GLM procedure. Means were separated according to Tukey's Mean Separation Test.

Results

Solvent type significantly influenced the migration of ϵ -caprolactam from the lidding material at the time of sealing, but there was no effect of solvent during subsequent storage at 20.6°C (Table 2). Epsilon-caprolactam concentration in 12% ethanol was 4.65 ppm immediately after sealing and at the same level (above 4 ppm) during storage. Concentrations of ϵ -caprolactam in the water samples indicated essentially no migration at the time of sealing or over the 28-day sampling period.

Simulated transport did not increase the migration of ϵ -caprolactam into either solvent (Table 2). Concentrations in the 12% ethanol were 4.31 ppm at the time of sealing in the non-transported cups and 4.76 ppm in the cups sampled immediately after the random vibration testing. Caprolactam levels were the same during subsequent storage, regardless of transport treatment or solvent except at 14 days, when cups subjected to simulated transport and containing 12% ethanol were significantly higher in ϵ -caprolactam than cups containing 12% ethanol which were not “transported”.

Results indicated that placement of the boxes within the shipping stack did not affect the migration of ϵ -caprolactam from the lidding material to the solvents (Table 3). Concentrations of ϵ -caprolactam in the 12% ethanol were ~4.76 ppm at all three locations immediately following simulated transport and remained at these levels over 28-days subsequent storage.

Table 2. Concentration (ppm) of ϵ -caprolactam in water or 12% ethanol immediately after sealing and during subsequent storage.

	Days Storage at 20.6°C			
	0	7	14	28
<i>Non-transported</i>				
Water	0.00 Aa ^{zy}	0.29 Aa	0.11 Aa	0.00 Aa
12% ethanol	4.31 Bb	5.95 Bb	5.36 Bb	5.38 Bb
<i>Simulated transport</i>				
Water	0.01 Aa	0.00 Aa	0.00 Aa	0.00 Aa
12% ethanol	4.76 Bb	3.89 Bb	3.67 Cb	4.02 Bb

^z Within a transport method, means within a row with the same lower-case letter are not significantly different at 95% confidence level according to Tukey's Means Separation Test.

^y At each day of storage, means within the column with the same upper-case letter are not significantly different at 95% confidence level according to Tukey's Means Separation Test.

Table 3. Concentration (ppm) of ϵ -caprolactam in solvent based on position of box within stack.

Solvent	Location	Days at 20.6°C			
		0	7	14	28 ^z
Water	Top	0.00 Aa ^{yx}	0.00 Aa	0.00 Aa	0.00 Aa
	Middle	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa
	Bottom	0.02 Aa	0.00 Aa	0.01 Aa	0.01 Aa
12% ethanol	Top	4.77 Bb	3.96 Bb	4.26 Bb	3.35 Ab
	Middle	4.76 Bb	3.54 Bb	3.42 Bb	4.56 Ab
	Bottom	4.75 Bb	4.17 Bb	3.34 Bb	4.14 Ab

^z At 28 days storage, means with the same upper-case letter were not significantly different at $Pr > F = 0.055$.

^y Within solvent, means within a row with the same lower-case letter are not significantly different at 95% confidence level according to Tukey's Means Separation Test.

^x Within a solvent, means within a column with the same upper-case letter are not significantly different at 95% confidence level according to Tukey's Means Separation Test.

Discussion

Results indicated that ϵ -caprolactam was migrating from the inner Nylon-6 layer of the lidding film across the PP layer into the alcohol solvent at the time of sealing. Migration of ϵ -caprolactam to 15% ethanol was observed from Nylon-6 granules and monolayer Nylon-6 films (Pogorzleska et al., 2001). Concentrations of ϵ -caprolactam ranged from 6.0 – 16.4 mg/kg of solvent; however, the testing protocols were significantly different than those used in this study. Pogorzleska et al. (2001) exposed Nylon-6 granules and Nylon-6 films to temperatures above 100°C for extended periods of time (up to 1 hour) and the plastic was in constant contact with the ethanol. In contrast, the results from the current study indicated that migration can occur very quickly, at lower temperatures, and that contact of the lidding material with the solvent was not necessary.

Due to the hydrophilic nature of ϵ -caprolactam, movement from Nylon-6 to water can occur easily (Barkby et al., 1993), especially when the water is in direct contact with the plastic. However, no published studies have been conducted to investigate the effect of headspace on the migration of ϵ -caprolactam to water. The presence of a ~1.5 cm headspace appears to have been sufficient to prevent the movement of ϵ -caprolactam. Only very small amounts of this compound migrated to water in the cups, even after a storage period of 28 days.

Gramshaw et al. (1998) determined that differences in migration of ϵ -caprolactam to roasted chicken varied depending on contact of the chicken with the plastic roasting bag. Higher levels were reported in the flesh and skin that was in direct contact with the plastic, while areas that were not in contact had significantly less ϵ -caprolactam. The

results of the present study support the observation of Gramshaw et al. that a space between plastic and solvent can greatly reduce the migration rate for ϵ -caprolactam.

Heating Nylon-6 plastics can significantly increase the rate of migration of ϵ -caprolactam (Barkby et al., 1993). However, Ramanathan (2007) found that long-term ambient storage (up to 64 weeks) of water in Nylon-6-based bags resulted in elevated levels of ϵ -caprolactam. In addition, Ramanathan (2007) conducted a taste test to determine the organoleptic impacts of ϵ -caprolactam in water. A panel found that a concentration of 12.6 ppm ϵ -caprolactam was acceptable and that no “off flavors” were detectable from the water.

In the current study, the levels detected in the 12% ethanol, ~4.75 ppm, were less than half the levels reported by Ramanathan (2007). Both studies support the observation that even though ϵ -caprolactam has been known to cause “off-flavors” in some solvents, more research must be done to test the organoleptic impacts of ϵ -caprolactam in alcohol, and, especially, wine, as some wine is packaged in plastic-lined cartons for distribution and retail sale.

Conclusions

In this study, there appears to be an interaction between the ethanol and the Nylon-6 lidding material, though this interaction is not understood. Little migration occurred into water at the time of sealing though ϵ -caprolactam is highly hydrophilic. The lidding material was placed on the cups immediately before sealing so there was very little time for the ethanol to interact with the film, and simulated shipping and storage up to 28 days at 20.6°C had no effect. Future work should therefore investigate the interaction of ethanol concentration, and sealing time and temperature, on the potential migration of ϵ -caprolactam from Nylon-6 lidding materials.

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