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EFFECT OF PACKAGE CONFIGURATION ON BARRIER PROPERTIES AND SENSORY PERCEPTION OF FLAVOR

presented by

PANKAJ KUMAR

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Michigan State University pherical comments in partial fulfillment of the requirements for the degree of

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ABSTRACT

EFFECT OF PACKAGE CONFIGURATION ON BARRIER PROPERTIES AND SENSORY PERCEPTION OF FLAVOR

By

Pankaj Kumar

The effect of processing on barrier properties of thermoformed containers was studied. Two containers with the same surface area, one a semi-spherical shape and the other a polyhedron shape with six corners, were designed and thermoformed with polypropylene resin. Crystallinity of the polymer in various parts of the containers was measured. The transmission rates of these containers to oxygen (OTR) and water (WVTR) were determined. These properties were studied in tandem with change in sensory properties of a simulated drink. A beverage system with benzaldehyde as flavor compound was created and stored at 23 °C and 50% RH for 3, 7, 14, 21 and 28 days in the two types of container. The drink was served to a trained panel to assess the flavor strength and was analyzed with HPLC to quantify residual benzaldehyde. WVTR and OTR of the polyhedron containers were 21% and 60%, respectively more than semispherical containers. Sensory studies and HPLC results showed stronger benzaldehyde character in semispherical containers after 21 days onwards. This study confirms that there is a relationship between package configuration and barrier properties of containers causing difference in the sensory flavor perception.

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To all souls who tried to make it better

had a question was Dr. Bruce Hore. I took at his personality and I always try to emulate his simplicity and like him, I try to keep my feet grounded. Though I took only one class with him, I think I leanut lessons worth a lifesing from him.

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One person who was not in my committee but was always available whenever I had a question was Dr. Bruce Harte. I look at his personality and I always try to emulate his simplicity and like him, I try to keep my feet grounded. Though I took only one class with him, I think I learnt lessons worth a lifetime from him.

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Pankaj Kumar

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1.1 Background

Polymers are fast growing into as preferred choice for packaging food and beverage products due to their competitive advantages such as customizable barrier and physical properties, and appearance and light weight. The ever increasing marketing requirements for a longer shelf life make it ever more challenging for packaging engineer to meet the expectations. In this regard, metal and glass have been packaging engineer's best friends for a long time, but now polymers and polymer based packaging systems have taken the center stage in packaging of food and pharmaceuticals. However, polymers, unlike metal and glass, are semi-crystalline materials and their properties such as morphology and barrier are subject to change during their processing stage and usable life. Since barrier properties dictate the shelf life in most food packaging applications, changes on them will affect the product performance. Extensive and rigorous research has been conducted to determine these properties and to assess different polymer barrier properties [1-7] and novel materials are being developed wherever deemed needed [8-10].

Polymers are vastly used now-a-days to package beverage products. The beverage industry has always been an intensely competitive market. New and enhanced products keep on appearing on a regular basis, and the focus has constantly been shifting on younger consumer generation. The products are not only carrying nutritional values these days but are more palatable and lasting. A lot of innovation goes in to improving flavor profile and stability of products making complex flavor systems take over the main stage.

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Such innovative and complex products need excellent protection to preserve the delicate flavor balance. This delicate flavor balance is intended to be preserved for the whole shelf life. One of the major causes of spoilage is mass transfer that includes exchange of moisture, oxygen and flavor compounds between the inside and external environment of a package. There are different approaches used alone and in combination with each other to extend the acceptability and shelf life of packaged food products. Incorporating food preservatives, improving package performance and controlling the storage environment are major approaches. The incorporation of preservatives like Butylated hydroxyanisole (anti-oxidant) is limited because of regulation on concentration of many of preservative ingredients. Besides, some of them like ascorbic acid (anti-oxidant and anti-microbial agent) have a flavor of their own, and thus they have to be used in sensible concentrations so as not to influence the desired flavor balance too much. In addition, these preservatives are not intended for reducing loss of flavor compounds and moisture. Sustaining a controlled environment in storage and in transit involves huge cost factor. Thus, it becomes imperative for the container to work beyond the function of containment and provide mandatory protection to the food product.

As suggested earlier, polymeric materials are vulnerable to changes because of processing. Change in orientation and crystallinity are prime examples of such changes occurring in the polymer matrix [11-14]. To the best of the author's knowledge very limited studies look into the barrier changes occurring during conversion from one format to another. For instance, what happens when the polymer is processed from resin to sheet form, or what changes occur when resin is formed in to bottles or when the sheet is thermoformed into containers. There have not been enough studies concerning how these properties change as a result of number and severity of processing stages. Some times, the polymers are rendered inappropriate because of inadequate processing conditions bringing in undesirable changes in the polymer matrix. This hinders the applicability of a polymer in a particular scenario and the packaging engineer ends up selecting a higher cost material or higher thickness of material which are not cost favorable.

Another issue overlooked is differences in container shapes. When same material is processed in two or more different container shapes or configurations, the ultimate barrier properties achieved of the containers might be different or might not be different. In this regard, there has not been any significant research. Possibility of barrier properties being affected differentially can not be ignored in the light of differential stresses being created because of geometry of the containers. This can cause morphological changes including but not exclusively crystallinity, distribution of crystallites, orientation etc. Looking into this might be of paramount importance, especially for flavor intensive products where the flavor concentrations used are minute.

As discussed above, barrier of containers might be affected by the processing based upon the container shape or configuration. If there is such change in the barrier properties, it would be of any importance to the food industry only if it is significant enough. In other words, if processing into different shapes is affecting the barrier of a polymer, it should be large enough to be detected by human sensory organs. For example, if the permeation rates of a flavor compound out of two different containers are different enough to be detected by the instrumental techniques but are not perceived differentially by human taste buds, then this difference is not of practical significance for food or other flavor-centric industries as its effects over marketability of the products would be null or very limited. In such a case, enhancing packaging would result in increase in cost without any change in consumer preference, ultimately resulting in losing profitability. In case of pharmaceutical or medical industry, where the functionality of compound is more important than the sensory perception of the product, enhancing packaging might still be required for the compounds that are included for their medicinal qualities, but they also carry flavor value. Benzaldehyde is one such highly aromatic compound that is commonly used in cough syrups [15]. On the other hand, if the flavor loss is different to a great deal so that consumers are able to perceive it, this would greatly affect the preference of consumers as well as shelf life of the product. The concentration of flavors used in various food beverages is very low to start with, and any significant loss can seriously damage the delicate balance. In such a case, it becomes imperative to move towards better design principles to ensure appropriate protection of permeating components. Thus, it becomes mandatory to study the possibility and extent of changes in barrier properties of the polymer as a result of processing it in to different shapes.

Chapter 2

1.2 Hypothesis

The main goal of this work is to assess the extent of impact on the barrier properties of containers affected by configuration or shape. The null and alternate hypothesis of this work can be expressed as follows:

Ho:

Two polymeric containers made with same material and same surface area but in different shapes have the same barrier properties and the product stored will have the same sensory flavor perception.

Hallenging by the day. If the package is not able to passed the product for its aroma

Two polymeric containers made with same material and same surface area but in different shapes have different barrier properties and the product stored will have different sensory flavor perception.

regulating agencies impose a penalty on the food exapony, but if a product loses its flavor or palatability, the consumer rejects the product and this same use to be a much bigger penalty for the food company. Thus, if becomes exceeded for the packaging engineer to look at the finer aspects of flavor/ packaging interactions. The exceeded factor that compounds the problem is that flavor are just 10⁻¹⁶ to 10⁻¹⁶ when the food composition [17]. More over, hundreds of compounds are requested for the part and particular product. Even though, the character impact compounds are neglected in two but loss of any of those flavor compounds alters the flavor profile, and the product are not be able to win consumer's preference. In this context, one of the more contextence are In that of finds the end of the Chapter 2 should be finds and finds paced flower benefits to very competitive, but also with the introduction of inneveries products Literature Review

2.1 Introduction

Food has two important aspects: nutrition and flavor. While nutrition makes the food essential, flavor makes it palatable. All the five human senses are involved in judging the flavor of foods. Aroma, taste, texture, visual appearance and even the sound are essential parts of the flavor [16]. With the introduction of more volumes and varieties of food products in packaged form, the job of packaging engineer is getting more challenging by the day. If the package is not able to protect the product for its aroma (volatile permeation), taste (lipid and microbial deterioration), texture (moisture uptake/ removal), visual appearance (non-enzymatic/ enzymatic browning etc.) or sound (crispiness, affected mainly by moisture content), the onus is on the packaging scientist. If a product loses the nutritional content and that goes below the label claim, the regulating agencies impose a penalty on the food company, but if a product loses its flavor or palatability, the consumer rejects the product and this turns out to be a much bigger penalty for the food company. Thus, it becomes essential for the packaging engineer to look at the finer aspects of flavor/ packaging interactions. The crucial factor that compounds the problem is that flavors are just 10^{-14} to 10^{-8} % of the food composition [17]. More over, hundreds of compounds are responsible for flavor of a particular product. Even though, the character impact compounds are just one or two but loss of any of those flavor compounds alters the flavor profile, and the product may not be able to win consumer's preference. In this context, one of the most challenging areas

is that of fruit flavors. Not only the slow growing market for fruits and fruit juice/ flavor based products is very competitive, but also with the introduction of innovative products like cherry, vanilla and grape flavored carbonated drinks, the challenge to packaging industry has become more incisive.

There are many facets to flavor/ packaging interactions: sorption, permeation and migration. All play specific roles in altering the flavor profile of a product making it difficult to keep it acceptable for the intended storage period (called shelf life). The sorption of aromatic compounds by the polymeric packaging materials can cause unbalanced flavor profile [18]. After the sorption of the flavoring compound, the polymer behaves as a channel for the flavor molecule to diffuse into, and finally liberate itself into the environment by being desorbed on the other end of the polymeric package. Desorption of undesirable flavors from polymers can also take place if the materials/ packages are being reused as was observed with PET refillable bottles [19]. Thus, these flavor compounds become part of the packaging material in the virgin cycle while they migrate to the product in the second and later cycles even though they were not intended or expected as additives or migrants. Such an event involves participation of the entire spectrum of mass transfer phenomenon. Research is going on in all the three aspects of flavor/ package interactions, and this review tries to bring together the fundamentals as well as recent developments in the field of mass transfer, factors affecting mass transfer. techniques to measure it and available data for important fruit flavors.

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2.2 Mass Transfer Phenomenon

2.2.1 Diffusion

Diffusion is the process of movement of substance within itself or another substance [20]. Crank [21] defined it as the process by which matter is transported from one part of a system to another as a result of random molecular motions. In context of diffusion through polymer, it involves movement of flavor compounds, moisture vapors, gases and additives across the polymer matrix. This phenomenon is mainly governed by the Fick's first law [22] at steady state:

$$F = -D(\delta c / \delta x) \tag{1}$$

where F is the flux of mass,

D is the Diffusion Coefficient,

 c_{affect} $\delta c/\delta x$ is the partial of concentration gradient and distance D, the average effective diffusion coefficient can be defined as:

$$D = \frac{1}{C_2 - C_1} \int_{C_1}^{C_2} \frac{D_{loc}}{1 - \omega} dC$$
(2)

where C_2 and C_1 are concentrations of the diffusant on the two sides of the polymer wall

 D_{loc} is the binary mutual diffusion coefficient of the diffusant in the polymer ω is the mass fraction of the diffusant in the polymer

Fick's law identifies concentration gradient to be the only driving force and such diffusion is called **Fickian diffusion**. It has been observed that sometimes, besides concentration gradient, other factors like gradients in stress or temperature might also drive diffusion. Also, concentration itself besides concentration gradient affects diffusion. Such a type of diffusion is called **Non-fickian diffusion** and can not be explained with Fick's laws of diffusion. In such cases, concentration dependence of diffusion is explained by two mechanisms: Flory-Huggins [23] and clustering [24]; wherein the first mechanism accounts for increase in diffusion coefficient, the other explains decrease in diffusion coefficient.

Diffusion plays an important role in determining the permeation rate of a permeant through a barrier material. This is a phenomenon that is active during both steady state as well as unsteady state. In simpler words, large diffusion coefficient means easy movement of permeant through the polymer wall and thus increasing the permeability coefficient values.

2.2.2 Sorption

Sorption is the uptake of product components, such as moisture, flavor, aroma or colorant compounds by the polymeric packaging material [11]. These components are called sorbates. This phenomenon is also referred as negative migration since the polymer behaves as the receptor of the sorbate instead of being source of the contaminant. Sorption is primarily governed by similarity between the polymer and the sorbate, chemical or polar. This is in fact related to solubility of the sorbate into the polymer. Mixing of a compound such as oxygen or an aroma vapor (a gas) with a polymer (solid) (or a liquid for that matter) at molecular level is called a solution [11]. At low concentration levels, it behaves like an ideal solution and follows Henry's law:

$$c_i = S \cdot p_i \tag{3}$$

where c_i is concentration of the solute (sorbate)

S is Henry's proportionality coefficient called Solubility coefficient

p_i is the equilibrium vapor pressure of the solute (sorbate)

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The solubility coefficient defines the rate at which a sorbate would get absorbed by the polymer or in other words what would be the extent of sorption. But Henry's law holds only when there is no interaction between the polymer and the sorbate. Henry's law works very well with O₂ and N₂, at essentially all pressures of practical interest but many organic vapors including many flavor compounds and CO₂ tend to deviate from Henry's law at sufficiently high pressures [11]. Langmuir- Henry's law equation is useful to describe high pressure permeant sorption in polymers like that of CO₂ in Polyethylene Terephthalate (PET) bottles. Other equations like Flory-Huggins equation [23] are more appropriate to use when there is an interaction between polymer and permeant. It defines the solubility relationship by including an interaction parameter correlating the vapor activity of the solute in the gas phase with its volume fraction in the polymer.

Whereas the solubility coefficient defines the rate of sorption at which the sorbate would be absorbed into the polymer, the partition coefficient defines the overall sorption that would occur. **Partition coefficient** is the ratio of concentration of the sorbate at equilibrium in two phases, here product and polymer phases.

$$Partition \ coefficient = \frac{[C_{eq}]_I}{[C_{eq}]_{II}} \tag{4}$$

where numerator and denominator denote the equilibrium concentrations of the sorbate in the two phases. Both solubility and partition coefficient are temperature dependent.

2.2.3 Permeation

"Permeation is the movement of gases, vapors, or liquids (called permeants) across a homogenous packaging material, and excludes the travel of materials through perforations, cracks, or other defects" [11]. Thus, this is the transport phenomenon of a permeant molecule from one side of the polymeric wall to the other side, from higher concentration/ pressure side to the lower concentration/ pressure side. Permeation is related to permeability or permeability coefficient, which is a characteristic of a barrier material for a specific permeant at a specific temperature. Permeability thus is independent of thickness of the barrier, partial pressure difference, area of exposure and exposure/ permeation time. All these variables are normalized in the following equation for permeability coefficient:

$$P = \frac{Q \cdot l}{t \cdot A \cdot \Delta p} \tag{5}$$

where P is Permeability coefficient at steady state

Q is total quantity of permeant that permeated through the polymer wall

1 is the thickness of the polymer wall

t is the total time of exposure/ permeation

 Δp is the partial pressure gradient between the two sides of the polymer wall.

Thus, permeability coefficient is a function of polymer, permeant and temperature. Steady state permeability coefficient can also be expressed as a function of Diffusion (D) and Solubility coefficients (s).

$$P = D \cdot S \tag{6}$$

The above equation holds at low concentrations of the permeant and when there is no interaction between permeant and polymeric material. Materials change behavior when environmental temperature changes, and in some cases materials change behavior when environmental relative humidity (RH) changes. Thus, permeability of a material for a particular permeant, changes as the temperature changes and sometimes is also affected by the change in environmental RH.

2.2.4 Migration

Migration is the phenomenon of polymer additives and components moving in to the product matrix. Examples of compounds that can migrate include residual monomers, solvents, catalysts, additives and even residual flavor compounds from the earlier polymer cycle if the polymer is being reused. Migration affects not only the organoleptic qualities but also the toxicological attributes of the food product. This can cause serious health problems as well as damage to the flavor profile. The flavor change caused by such migration of polymer components is deemed as off-flavor.

2.3 Permeability Measurement

Permeability can be measured using many different techniques. Isostatic and quasi-isostatic, gravimetric techniques are the most popular techniques. These techniques are more or less applicable depending on scenario. These techniques are briefed below.

2.3.1 Isostatic technique

Isostatic technique involves maintaining the partial pressure gradient of permeant on the two sides of the packaging material constant. The high pressure side is kept constant while the low pressure side is maintained at zero in most cases. This is achieved either by sweeping a carrier gas or a trapping agent. Diffusion and permeability coefficients can be measured with the following equations, once the steady state flow has been established.

tate marked by constant rate of mass transfer

$$D = \frac{l^2}{7.2t_{1/2}}$$
(7)

$$P = \frac{Fss \cdot l}{\Delta p} \tag{8}$$

where 1 is thickness of the barrier permeability

Fss is the steady state flux

 Δp is the partial pressure difference

 $t_{1/2}$ is the time to achieve half of the steady state mass uptake

Solubility coefficient can be calculated once values for P and D are available using the Fick's law. It is a common practice to use this technique to measure the permeation of generally non-interacting permeants like moisture vapor, O_2 , CO_2 and N_2 [25-27]. There have been attempts to measure organic vapor permeability too using this technique [7, 28] but it becomes essential to conduct a consistency test to make sure that the results are not adversely affected by the interaction between the permeant and the packaging material. Permeability equipments like Mocon Permatran, Oxtran, Illinois instruments utilize this technique. ASTM E96, F1249 and D3985 are also based on this technique.

2.3.2 Quasi-isostatic technique

Quasi-isostatic technique is applied in scenarios where it is not feasible to continuously keep the low pressure side at zero and detect the permeant simultaneously. This is a pressure-variable method. The diffusion coefficient is calculated by lag time (θ) , which is the time required for the particular permeant- polymer system to reach steady state marked by constant rate of mass transfer. 2.3.3 Gruvimetric Technique

$$D = \frac{l^2}{6\theta}$$

$$P = \left[\frac{q}{t}\right]_{ss} \frac{l}{A \cdot \Delta p} \tag{10}$$

(9)

where 1 is the thickness of the barrier material

 θ is lag time

 $(q/t)_{ss}$ is the slope of the accumulated quantity of permeant plotted against time at steady state

A is area of barrier material

Δp is the partial pressure gradient

The quasi-isostatic method involves change in pressure gradient (usually below 5%) but still assumes the changes to be effectively small enough to assume changes in Δp to be zero. The apparatus for the quasi-isostatic technique involves use of a permeability cell with two parts separated by the barrier material. The low pressure side has a sampling port used to sample the headspace that is analyzed to quantify the vapor of interest. This method is heavily utilized to measure the permeability of organic vapors individually as well as in combination to each other [29-31]. A gas chromatogram is typically utilized for separation and quantification with a Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD) or Mass Spectrophotometer (MS) detector. This gives an opportunity to measure permeability of two or more organic vapors in tandem, bringing it closer to real field conditions.

form or another to measure permeation rates. The development is small, it was to the

2.3.3 Gravimetric Technique

Gravimetric technique is considered as a type of isostatic technique, but it has grown as a very significant technique [25, 32, 33] and deserves a separate mention. With this technique, the permeability coefficient is calculated indirectly by measuring solubility and diffusion coefficients. The polymer is exposed to the vapors of interest, and the weight change is observed at very short time intervals. The solubility coefficient is calculated from the total sorption by the polymer once the steady state is achieved. The diffusion coefficient is calculated with the data available for non-steady state. For noninteracting permeants, in general, following gravimetric diffusion coefficient is calculated by the following equations: $D = \frac{l^2}{7.2t_{1/2}}$ (11) $S = \frac{W_f - W_i}{W_c \Delta p}$ (12)

where W_f is the weight of the polymer after attaining steady state

W_i is the dry weight of the polymer

The product of D and S provides the permeability coefficient. Exciting developments have taken place with this technique with novel kinds of detectors and microbalances utilizing it for water and organic vapor permeation measurements.

2.3.4 Miscellaneous Techniques

Most of the novel techniques utilize isostatic or quasi-isostatic dynamics in one form or another to measure permeation rates. The development is usually limited to the detector and the techniques to derive information from the varied kinds of detectors. One of the exciting advances is the utilization of Quartz Crystal Microbalance (OCM) to measure the permeability of extremely thin films [34-39]. The mass uptake by the polymer film is measured by change in resonance frequency of the quartz crystal which has been coated with the polymer film. Once the mass uptake has been calculated, the same equations as for the gravimetric technique are utilized to calculate the mass transfer coefficients. Another technique of importance is Fourier Transform Infrared (FTIR) spectroscopic measurement. With this method, the variable being measured is the particular IR wavelength that is characteristic of the permeant compound. This has proved to be an important tool when the need is to study permeation in a dynamic environment in tandem with some other effects [40-44]. Small Angle Neutron Scattering (SANS) is an upcoming technique being applied in some studies [45, 46]. A big advantage with this technique is that it not only helps understand the diffusion process, but also lets to look at the morphological changes including glass transition temperature and d-spacing between the polymeric chains, which is defined as the perpendicular distance between two planes inside a crystal.

2.4 Parameters governing permeability

There are many factors that affect the permeability of packaging materials towards moisture, gases and organic compounds. Permeation occurs mainly in two stages, unsteady state and steady state. During the unsteady state, the rate of concentration change of permeant across the matrix of the polymer is variable and gradually increasing while it becomes constant in the steady state. Diffusion and sorption both play significant role in the unsteady state while diffusion takes over and sorption

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reaches dynamic equilibrium during the steady state. Both sorption and diffusion are affected by myriad factors that can mainly be classified originating from 4 sources: Polymer, permeant, affinity between polymer and permeant, Environmental conditions. Because of such multiple factors, every permeation phenomenon becomes unique and attention has to be paid to each individual element in order to understand it. Figure 1 depicts the process of permeation and the factors affecting it. This "wheel of permeation" provides a snapshot of the factors involved during permeation.



Figure 1 Wheel of Permeation

oriented polymer. The orientation of polymer brings the chains above an energy energy

2.4.1 Polymer them. They density increases and the molecular mobility in oriented

2.4.1.1 Crystallinity

Crystalline materials are known to be impervious to gases, moisture and organic compounds. Metal and glass are perfect example of that. Polymers being semi-crystalline are affected by the extent of crystallinity present in the polymer. It has been shown that crystallinity has a bearing in the effectiveness of a polymer as a barrier [4]. In the crystalline part of a material, the mobility of a permeant is very limited, and it is considered that all mass transport through semi-crystalline materials is through the amorphous regions [11]. It has been also suggested that the mass transfer rate is highest through the interfacial regions between crystalline and amorphous regions owing to the enhanced stresses in those areas [11].

2.4.1.2 Distribution of Crystallites

Distribution of crystallites affects the permeability in a unique way. An appropriate distribution of crystallites presents a tortuous path of diffusion to the permeant. Permeant thus require higher activation energy to diffuse through such a polymer. Same reasoning is applied to create novel barrier material by utilizing nanomaterials based on various kinds of crystalline materials. In such composite materials also, getting an appropriate dispersion is very important to achieve optimal properties. Besides the crystallites present in the polymer chain restrict the movement of amorphous parts and thus increasing the activation energy and barrier against permeation [47].

2.4.1.3 Orientation

Random and disorderly polymeric chains do not provide equivalent barrier as an oriented polymer. The orientation of polymer brings the chains closer to each other as

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well as aligning them. Thus, density increases and the molecular mobility in oriented regions decreases. This decreases the permeability by limiting diffusion of the permeant through film. This is a generally accepted argument, but there have been some anomalies reported to that as well. One such reported behavior was that of Vinyl Dichloride copolymer that showed increased permeability to oxygen when biaxially oriented as compared to unoriented extrusion cast film [48]. The researchers attributed the increase to microvoid development in the polymer matrix caused by orientation.

2.4.1.4 Free Volume

Free volume represents the volume available in the polymer matrix that is not increment from the increment parter of concentration the nertice, occupied by the polymer chains or additives. These are the gaps created in between the entangled polymer chains and thus are available for the permeant to occupy and provide a channel for diffusion. Thus, availability of more free volume means both more diffusion and solubility. This mainly relates to the density of the polymer [49]. Less density would mean more free volume and thus more permeability. This further can be related to molecular weight and its distribution. As the molecular weight distribution increases, the sorption increases [11, 50]. This probably can be explained on the increased irregularities in the polymer matrix providing more channels and voids at the surface for the permeant to invade. In this context LLDPE which has nerrow molecular weight distribution is known to be a better barrier compared to LDPE which has relatively broader molecular weight distribution. The density of a polymer also works against sorption [49] and ease of diffusion.

2.4.1.5 Cohesion & Adhesion Forces

Secondary forces between the polymeric chains play a critical role in defining the barrier of a polymer. For example, polyvinyl chloride (PVC) being completely amorphous would be expected to be a poor barrier. On the contrary, unplasticized PVC is an excellent barrier to gases, organic vapors and moisture. The partial charge on chlorine and hydrogen atoms that allows making H-bonds and thus providing secondary forces between polymer chains, restrict the chain mobility. That raises the glass transition temperature and the activation energy, and limits the diffusion and permeation. Similarly introduction of metal ions in polymer chains like that of LDPE raises its barrier properties as evident from the increased barrier of ionomers like surlyn.

2.4.1.6 Additives

Additives usually behave as plasticizers increasing the chain mobility and thus permeability. Additives can decrease the free volume available but this effect is expected to be very small because of the small quantities used. This effect is also nullified by the very nature of the additives themselves. Many additives have inorganic parts that create stresses at interface and cause more free volume in these regions thus aiding further in the permeation process. Those additives behave like impurities breaking continuity of otherwise continuous phase. There are also some additives that might act as bridges between the polymeric chains and in such a case they limit the permeability by enhancing the intermolecular forces and reducing the mobility of polymer chains [51].

2.4.2 Permeant

2.4.2.1 Molecular Size & Weight

Smaller and lighter molecules can diffuse easily and fast, simply because they require less amount of energy to move [11]. Besides, they can reside in smaller spaces as compared to bigger molecules, and can travel through narrow channels and defects available in the polymer matrix. This is the reason that H₂ and He are the most permeable permeants through all non-impervious materials.

2.4.2.2 Vapor Pressure

Vapor pressure of the permeant makes it more or less mobile at room temperature. This relates to molecular weight and boiling point of the permeant. The molecules of gases are very mobile because at room temperature they are in gaseous phase and thus exhibit high diffusion. Other viscous and high molecular weight permeants need relatively higher amount of energy not necessary available abundantly at room temperature. The permeants exhibit relatively lower diffusion capability. The vapor pressure of a particular permeant would also change depending upon what kind of system it is trapped in. For example, different foods containing same flavor will have different partition coefficient with air for the same flavor compound. This would cause different vapor pressure of the permeant on top of the different food matrices. High vapor pressure also causes deviation from Henry's law of solubility, where Langmuir- Henry's law equation holds more relevance [11].

polyoients, make for better barner against moisture wayse with which is partial polynature stops the organic vapora to permeate flavough it while permeating being any plately apolar in nature stop a polar molecule like moisture to pass through the barry anothe 2.4.3 Affinity and the defendence are the fastest to get absorbed and permeate when in

2.4.3.1 Chemical Similarity

Chemical similarity between the polymer and the permeant increases the solubility of permeant in the polymer. For this reason, the long chain permeants have a greater solubility in polymers since polymers themselves are long chain organic compounds. The effect of carbon chain length is observed especially in case of aldehydes. Aldehydes in general show relatively low affinity towards polyethylene. But as the carbon chain length increases, the sorption also increases [49]. On the other hand such long chain permeants would have decreased diffusivity for the requirement of higher activation energy to mobilize the larger molecules. Similarly, same functional groups enhance the solubility as well. Such chemical similarity reduces the surface tension between the two phases and may also cause relaxation of polymer chain. If the affinity is very high, causing clustering, diffusion coefficient will go down. In all scenarios, chemical similarity will increase the solubility coefficient and depending upon the dominance of diffusion or solubility, the permeability coefficient may increase or decrease.

2.4.3.2 Polar Similarity

Polar similarity affects the surface tension between the polymer and the permeant and thus the solubility of permeant and the polymer. This is the reason that PET is relatively a better barrier against organic vapors while poly ethylene and other polyolefins make for better barrier against moisture vapor. PET with its partial polar nature stops the organic vapors to permeate through it while polyolefins being completely apolar in nature stop a polar molecule like moisture to pass through. Similarly straight
chain hydrocarbons like d-limonene are the fastest to get absorbed and permeate when in contact with LDPE while compounds like hexanol and linalool are relatively slow and less in extent given the presence of hydroxyl group. In this context, the affinity towards getting absorbed in an apolar polymer like poly ethylene has follows this sequence: hydrocarbons> ethyl esters> aldehydes> alcohols [49].

Enhanced solubility of long carbon chain permeants can be also explained on the relative contribution of the polar part which is decreasing as the carbon chain length increases in relation to the permeants that have non-polar functional groups [52]. The same phenomenon was observed with esters. For the polymers that are prevalently apolar in nature, such permeant would absorb and diffuse faster while in polymers that are relatively polar, shorter carbon chain would absorb and diffuse faster.

2.4.4 Environment

2.4.4.1 Partial Pressure

The partial pressure gradient defines the driving force for the permeation process. For permeants like O₂, it is relatively a universal gradient. For organic permeants, it would vary based on the product under consideration, or the specific environment.

2.4.4.2 Temperature

Effects of temperature on permeability are very well recorded. Generally, permeability increases with increasing temperature. As the temperature increases, polymer chains become more mobile as they get closer to the glass transition temperature (in case of glassy polymers) or further higher than it (in case of rubbery polymers). Besides, because of the increase temperature more and more permeant molecules now have the required energy to diffuse in and permeate through. The effects of temperature are best defined by an Arrhenius-type relationship: in metric [55]

$$P = P_o e^{-Ep/RT} \tag{13}$$

Temperature has two pronged effect on the permeability. One, it provides more energy to the permeant molecule to go through the polymer wall, and two, as the temperature goes up, the chains in the amorphous region of polymer become more mobile, thus allowing the permeant to pass through. In other words, the barrier of polymer goes down while permeant molecules have more energy to cross the barrier. But, there are a few anomalies. Polystyrene and polylactide have negative activation energy [53]. It is hypothesized that some kind of cross-linking activity takes place in polystyrene increasing its barrier at higher temperature. Polylactides exhibit regular barrier trend against oxygen and CO₂, but with moisture the trend reverses [53]. Temperature affects the partition coefficient also, thus affecting solubility. With increasing temperature the solubility usually decreases, but there are exceptions to it. The partition coefficients (polymer/ gas) of ethyl acetate, n-hexanal, and d-limonene at vapor activity of 0.2 were found to decrease as the temperature increased from 25 °C to 40 °C for barrier materials such as foil, metallized- PET (M-PET), SARANTM and ethyl vinyl Alcohol (EVOH) [54]. In the same study it was found that partition coefficient (polymer/ gas) of α -terpineol with all the materials and SARAN with ethyl acetate increased.

2.4.4.3 Co-permeants

If there is a significant interaction between a permeant and the polymer, it is quite possible that the polymer would swell by absorbing the molecule. This would mean increased voids and spaces between the chains paving the way for other permeants to pass through. This phenomenon has been noted with d-limonene and ethyl acetate where d-limonene helps ethyl acetate escape from polyolefin matrix [55].

2.4.4.4 Relative Humidity

Relative humidity affects only those polymers that are hydrophilic meaning they have some functional group in the chain that can create hydrogen bonds. In most circumstances, as the relative humidity goes up the polymer's barrier towards other permeants goes down. EVOH is a classic example of such a polymer. Water molecules get themselves attached through H-bonding with the hydroxyl functional group and act as a plasticizer for the polymer making the chains further apart and increasing the permeability [50]. Thus, the permeability in this case is a function of not just temperature but relative humidity as well. In some cases, there have been claims of increasing barrier with increasing RH. Some trademarked amorphous type of nylons (Selar[™] from DuPont[®]) are claimed to exhibit such a property. It can be explained in terms of creating pseudo-crosslinks with limited H-bondings between the polymeric chains [56].

individuality and difference (this concerns specifically to synthetic flavorings)

2.5 Flavors

2.5.1 Flavor vs Off-flavors

Flavor is often defined as a sensation realized by the human brain when a food or beverage is placed into the oral cavity, and this is dependent upon the reactions of the taste and olfactory receptors to the chemical stimuli of the particular compounds present in the foods [57]. In oversimplified terms, flavor is the product of a physiological reaction that, defies objective measurement [58]. But this definition does not define the distinction between flavors and off-flavors. More effective definition will be "flavor substances are strong-smelling organic compounds with characteristic and usually pleasant odors" [59]. Thus, we can distinguish flavors and off-flavors as both belonging to the same chemical species category of compounds producing sensations through taste and olfactory receptors but flavors being pleasant and "in-place" while off-flavors being either unpleasant or "out of place" e.g. milk picking up onion smell because of being stored along with onions in refrigerator.

2.5.2 Flavor Constituents

Flavor is composed of three parts namely character impact items, contributory items and differential items. Character impact items are those which when smelled and/ or tasted are reminiscent of the named flavors and it provides most of flavor's organoleptic effect. Contributory items are those that when smelled and/ or tasted help to create, enhance or potentiate the named flavor. These bring the character impact items closer to the flavor. Differential items are additives and combination of additives that have no or little reminiscent of the named flavor and are added just to provide individuality and difference (this concerns specifically to synthetic flavorings).

2.5.3 Flavor Scalping

Flavor Scalping is defined as the loss of flavor compounds (mainly of character impact and contributory items) due to either absorption by the package or permeation through the package after the product has been packed. This causes flavor imbalance and thus loss of consumer preference. The sorption of flavor compounds by the packaging materials and into the seals might severely affect the polymer as well as overall package performance.

2.6 Flavor compounds of some major fruits and their thresholds

Presented below is a list of major flavor compounds of some fruits and their odor and flavor thresholds whichever is applicable. The list has been compiled by acquiring threshold values from mainly two great sources [60, 61] which are online database extracted from many resources with references provided. The flavor compounds found in different fruits have been from many different sources [57, 59, 61-74]

Important Constituents	Major Fruits	Odor threshold (ppb)	Flavor threshold (ppb)	
2,5-Dimethyl-4- methoxy-2H-furan-3- one	Strawberry	10		
2-heptanone	Raspberry	140-3000	1000	
2-methylbutan-1-ol	Apple	4000		
2-methylpropan-1-ol	Apple	7000		
2-methylpropyl acetate	Apple Raspberry	800		
2-phenylethanol	Apple	750		
3-methylbutan-1-ol	Apple	250-300	170	
3-methylbutanoate	Peach	60-76		
Benzaldehyde	Apple Cherry	350-3500	1500	
	Peach			
hay 2 anal	Amala	0.35		

Table 1 Major flavor compounds of some fruits and their threshold values in water

Hexan-1-ol

Important Constituents	Major Fruits	Odor threshold (ppb)	Flavor threshold (ppb)
Benzyl alcohol	Peach	10000	5500
	Raspberry		
Ethanol	Apple	100000	52000
Ethyl 2- methylbutanoate	Apple	.0061	0.1
Ethyl Butanoate	Strawberry	1	450
	Apple Raspberry		
	Maspocity		
Ethyl Butyrate	Grapes	1	450
Ethyl Hexanoate	Strawberry	3	
	Peach Raspherry		
	Kaspberry		
Eugenol	Cherry	6-30	
Geraniol	Cherry	40-75	
	Raspberry		
H-(4-hydroxyphenyl- butan-2-one) (raspberry ketone)	Raspberry	100	
hex-2-en-1-ol	Apple		
hex-2-enal	Apple	17	
hex-3-en-1-ol	Apple	70	
hex-3-enal	Apple	0.25	
Hexan-1-ol	Cherry	6	

Important Constituents	Major Fruits	Odor threshold (ppb)	Flavor threshold (ppb)
hexanal	Apple	4.5-5	16-76
	Cherry	4.5-5	
Linalool	Strawberry Cherry Blue Berry Peach Raspberry	6	
methyl anthranilate	Grapes	3	
nonanol	Peach	50	
Nona-trans-2,Cis-6- dienal	Cherry	0.1	
Octanal	Cherry	0.7	5-45
pentan-2-one	Apple	70000	
pentyl acetate	Apple	5-5000	3000-6600
Phenylacetaldehyde	Cherry	4	
p-Menth-1-en-9-ol	Cherry		
propyl butanoate	Apple	18-124	
trans-hex-2-en-1-ol	Blue Berry		
trans-hex-2-enal	Cherry Strawberry Blue Berry	17	
trans-Non-2-enal	Cherry	0.08-0.1	6

Important Constituents	Major Fruits	Odor threshold (ppb)	Flavor threshold (ppb)
a-ionone	Raspberry		0.4
a-terpineol	Peach	330-350	
β-damascenone	Grapes Raspberry	0.002	
β-ionone	Cherry Raspberry	0.007	
β-Phenylethanol	Grapes	750	
γ-decalactone	Raspberry	11	88
γ-dodecalactone	Peach	7	
γ-hexalactone	Peach	1600	
γ-undecalactone	Peach		
δ-decalactone	Peach	100	90-160
δ-dodecalactone	Peach		1000
δ-undecalactone	Peach	150	

Chapter 3

Materials and Methods

3.1 Introduction

The major stepping stones of this work were making containers, making the drink, developing analytical techniques and ultimately conducting the testing after filling and storage of the product in the containers.

As part of the preliminary work, the packaging material and flavor compound were selected. Polypropylene (PP) was chosen as the packaging material. PP is a very common polymer used in food and pharmaceutical industry. PP is also known to have very limited ability in protecting against flavor loss as it is a polyolefin, being of nonpolar nature like most of the flavor compounds. This gives the possibility to demonstrate the hypothesis in reasonable time frame and providing efficient tools for effective cost management with the use of a relatively cheaply available polymer.

Benzaldehyde was chosen as the flavor compound. Benzaldehyde is a character impact compound for cherry and almonds and is also known as bitter almond flavor. It is also found in many fruits of commercial importance including apples, peaches, plums and many types of berries. Benzaldehyde has both aromatic and taste qualities, and thus involves the use of all flavor sensing organs. Benzaldehyde also has medicinal effects and is also used in pharmaceutical industry [15, 75].

Two different package configurations were needed that would be different to each other in configuration and also practically feasible. A round semi-spherical shape and a polyhedron shape with six corners were used for this purpose. This allows comparison between two configurations, one with no corners and one with multiple corners. The designs were created so as to allow for equal surface area. This did not include the lidding part which was ensured to be a total barrier achieved by the use of foil based materials. Thermoformability of containers was also a consideration. Very complicated and intricate shapes were not feasible to produce with simple machines available inhouse.

Simulated cherry drink was created by starting with a simple composition of a fruity flavored drink based on an acid-sugar system. Gum acacia was added to the drink to make benzaldehyde (natural oil) soluble in the aqueous system and vitamin E was added to serve the purpose of anti-oxidant and fortifier. Appropriate levels of flavoring compound were found using threshold study for benzaldehyde concentration in the base system.

Design files for the molds were created for both the two shapes identified for the containers. For milling the molds, many parameters were defined when converting from a 3-dimensional design file to a machine understandable command file. These parameters were optimized by trial and error.

Polypropylene sheets were cast extruded. The optimal extrusion conditions were found to get appropriate thickness with least variability achievable with both thickness (measured after regular periods with a micrometer) and crystallinity (measured in terms of visual clarity of the plastic sheets).

The molds were used to thermoform the containers from the polypropylene sheets. Thermoforming conditions were optimized to provide maximum productivity with both types of containers. After the drink composition was finalized and the containers were manufactured, the product was manufactured and packed in clean conditions. High

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Pressure Liquid Chromatography (HPLC) technique was developed for quantification of benzaldehyde in the drink. Lid configuration and sealing conditions were identified.

The product was filled and sealed in the containers for the Phase I pre-run and stored under room environment at 23 °C and 50% RH. Sensory and HPLC evaluation was conducted after 2 and 7 days' storage. For the sensory evaluation, consumer panel was used and subjected to triangle test. The sensory studies were approved by Institutional Review Board (IRB) and the relevant documents are attached in appendix A.

Based on the experience of this preliminary run, many changes were made to the overall process. Individuals were screened for their ability to rate the strength of benzaldehyde in the drink. Selected individuals were trained for 5 weeks and so developed trained panel was used for final testing. For the final stage of testing, drink was prepared under nitrogenous environment. Product filling and sealing was carried out under nitrogenous environment also. Filled containers were stored in a nitrogen filled chamber for 3, 7, 14, 21, 28 days.

Testing at 28 days was replicated. Trained panel rated the concentration of benzaldehyde in the product, and HPLC was used to quantify the concentration. Permeability of the two containers for water, oxygen and benzaldehyde was also measured. Crystallinity in the walls and corners of the containers was also measured. Figure 2 provides schematics of the materials and methods section.





3.2 Simulated Drink

3.2.1 Drink Design

The process flow for drink design is attached in appendix B. In any natural drink system, there are few essential components and flavorings other than the identifying flavor. It usually constitutes of a sour-sweet system with anti-oxidants. The drink system that we used as a simulant had sugar as the sweetener and citric acid as the souring agent. α -Tocopherol acetate or Vitamin E served dual purpose of anti-oxidant and fortifying agent. Benzaldehyde being natural oil required an emulsifying agent to dissolve in water therefore gum acacia was used for the purpose. Food grade benzaldehyde (98.5% purity) and citric acid were procured from Sigma-Aldrich, St. Louis, MO. "Spartan" brand household granular sugar was provided by Spartan Stores Distribution, LLC, Grand Rapids, MI. Gum Acacia (grade: IRX 4765) used as emulsifying agent was provided by Colloides Naturels, Bridgewater, NJ. Vitamin E (grade: 500 BG) used as anti-oxidant was provided by BASF, Florham Park, NJ. The required concentration of benzaldehyde was defined after threshold testing (discussed later). It was decided to use 45.75 ppm as targeted concentration of benzaldehyde in this drink system. Vitamin E and gum acacia were used in accordance with the recommended usage levels by the manufacturer. Table 2 shows the concentrations of the various components used:

Table 2 Concentrations of various constituents of the drink

Component	Sugar	Citric Acid	Vitamin E	Gum Acacia	Water
Concentration (g/L)	84.55	1.06	2	0.66	Balance

This composition was tested for stability for benzaldehyde. The procedure and results of the stability test are presented in appendix C. The final drink was prepared by adding gum in water to give 6.6% gum-water solution. Benzaldehyde was added to the concentrated gum-water solution. The solution was stirred at ambient temperature until all benzaldehyde was dissolved and individual droplets were not visible. Citric acid, sugar and vitamin E were added and solution was stirred for some more time. The solution was then brought to the correct concentration by diluting it. The surroundings were sanitized by spraying 0.05% Sodium Hypochlorite solution. For the preliminary testing, the product was prepared under room environment. For the second phase of testing, the preparation was carried out in nitrogenous environment inside a small chamber to avoid possibility of oxidation of benzaldehyde.

3.2.2 Consumer Panel Benzaldehyde threshold

Threshold of benzaldehyde perception in this simulated drink system was found in accordance with ASTM E 679-04 [76] using a 5-series 3-AFC test. The test was run on SIMS[®] 2000 software provided by Sensory Computer Systems, Morristown, NJ in the Sensory Laboratory, Department of Food Science, MSU. For each of the 5 concentration steps, the consumers were served with approximately 15 mL of three samples in 60 ml identical cups coded with unique 3-digit codes. Out of the three samples, two were blank drink samples containing no benzaldehyde while the third sample had specific benzaldehyde concentration. Samples were provided in random sequence. Concentrations used were 4.9, 14.7, 44.1, 132.3 and 396.9 ppm. The threshold study used 30 consumer panelists that mainly were students, faculty and staff members of Michigan State University. The promotional flyer is attached in appendix D and consent forms are attached in appendix E. Panelists were asked to pick the odd sample out. The questionnaire presented to the panelists is presented in the appendix F.1. To calculate group threshold, first individual thresholds were calculated. Individual thresholds were calculated to be the geometric mean of the last concentration step at which the respondent could not identify the correct sample and the next higher concentration level. If a panelist identified the correct sample at all concentration steps, then it was assumed that he would have answered incorrectly at one step lower than the smallest concentration level. Group threshold was calculated as geometric mean of individual thresholds.

Individual Threshold (IT) =
$$(C_n \times C_{n+1})^{0.5}$$
 (14)

Group Threshold (GT) =
$$(IT_1 \times IT_2 \times ... \times IT_N)^{(1/N)}$$
 (15)

.....

where C_n is the highest concentration step at which the panelist gave an incorrect answer

 C_{n+1} is the next concentration step to C_n

N is the number of panelists

With this experimental design, it was found that the consumer threshold of benzaldehyde in this particular design was 15.25 ppm. The table of responses is presented in the appendix G. Based on the threshold value, as suggested in the earlier section, 45.75 ppm was used. Primary reasons were that this concentration lets enough allowance so that consumer could taste the flavor at various time stages, and it is also close to 44.1 ppm, the concentration in the threshold test at which people liked the product the most.

3.3 Molds

3.3.1 Designs

The mold designs were created using Rhinoceros[®] 3.0 provided by McNeel Corporation, Seattle, WA. The semispherical and polyhedron containers had an overflow volume of 100.2 mL and 81.3 mL respectively. The containers had equal surface area of 82.8 cm². The semi-spherical and polyhedron containers are shown in the figures 3 & 4 below:



The command codes for designing the molds are listed in the appendix H. The original file format of the mold designs corresponded to 3-dimensional design (.3dm) which was converted to mesh or stereo lithographic file format (.stl). This file format was used by the Deskproto[®] 4.0 provided by Delft Spline Systems, Utrecht, Netherland. In Deskproto[®] 4.0, milling parameters like mill tool, step height, free movement height were defined. These parameters are listed in the appendix I. Parameters were optimized for milling time estimate and fineness achieved. More milling steps would bring more fineness meaning less sanding later on while coarse milling required more sanding. Deskproto[®] 4.0 converted the design to a machine understandable file format (.ncm). This file format is essentially a command text file understood by the mill software ((CPmill[®]) used by the Minitech minimill/2 desktop CNC machine. Both the mill machine and the operating software were provided by Minitech Machinery Corporation, Norcross, GA, Figures 5 & 6 show the semi-spherical and polyhedron molds.



Figure 5 Semispherical Mold



Figure 6 Polyhedron Mold *All dimensions in centimeters

3.3.2 Production

Renwood procured from Huntsman Advanced Materials, East Lansing, MI was cut in blocks of $12.7 \times 12.7 \times 5.08$ cm. These blocks were used to make each mold. Milling operation of the molds took approximately 6-7 hours for each mold. The molds were then sanded using sandpapers of different fineness (#80 to #120). On the bottom side of the molds, channels were created for the suction of air. Vacuum holes that connected to the channels on the bottom side were drilled on the surface of the molds with #80 drill bit (diameter= 0.3429 mm) using air-drill.

3.4 Polypropylene Sheet Extrusion

Polypropylene resin (grade: PP4612 E2) was provided by ExxonMobil Chemical company, Baytown, TX. Polypropylene sheets with a thickness of 12 ± 5 mil were cast extruded using Killion KLB 100 cast film extruder manufactured by Davis-Standard, LLC, Pawcatuck, CT. The temperature of the three zones of the extruder was 400 °F (204 °C). The adapter and die temperatures were 410 °F (210 °C) and 390 °F (199 °C),

respectively. The melt temperature was 360 °F (182 °C). The screw speed was 75 rpm. The temperature and speed of the chill roll were maintained at 75° F (24 °C) and 85 rpm respectively. The rubber roll pressure was 30 psi (0.21 MPa), and the rewinder speed was 40 rpm.

3.5 Container Thermoforming

The containers were thermoformed on a Hydro-trim 1620 thermoformer manufactured by Hydrotrim Corp, Valley Cottage, NY. Prior to thermoforming, the molds were sprayed with Ultra4 anti-stick food grade mold release agent manufactured by Price-Driscoll Corporation, Waterford, CT. The polypropylene sheets were heated by keeping upper and lower heating platens at 420 °F (216 °C) so as to allow for optimal sagging. The average heating time was 45 seconds. The forming time was kept at 25 seconds, and the suction pressure was 40 psi (0.28 MPa). Because the starting polypropylene sheets had a variable thickness profile, the formed containers were visually inspected for appropriate formation, and then were accepted depending on the thickness profile and surface smoothness. Excessively thin containers and containers with very rough texture at sealing rims were rejected. The excess material was trimmed off the good containers and the containers were then stored at 23 °C and 50% RH.

3.6 Lid Configuration

The lidding material was composed of two layers of foil based materials. As shown in figure 7, one of them was a sealable foil that was supplied by Tolas Healthcare, Feasterville, PA. This sealable foil had universal sealing material coated on 1.1 mil Aluminum foil making it sealable with polypropylene and also providing for peelability.

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The food side layer was a 3 mil adhesive backed foil provided by Uline, Waukegan, IL. It was introduced in the lidding structure in order to prevent contact of sealant material with the beverage. For semi-spherical containers, the inner material was $8 \times 8 \text{ cm}^2$ while the sealing material was $12.5 \times 10 \text{ cm}^2$. For polyhedron containers, the inner adhesive backed material was $8 \times 11.25 \text{ cm}^2$ while the sealing material was $14 \times 10 \text{ cm}^2$.



Figure 7 Lid configuration

3.7 Shelf Life Studies

3.7.1 Phase I: Pre-run Testing

3.7.1.1 Filling

The filling of the containers was carried out in a sanitized environment. The sanitizer was produced by diluting Clorox (6% Sodium Hypochlorite) manufactured by The Clorox Company, Oakland, CA so as to get at least 100 ppm active chlorine evolution. This was achieved by adding at least 2 mL of Clorox in 1 L of clean water. Sanitizer was sprayed all around the working area. The product was filled in such a way to have minimal headspace without interfering with the sealing area. Average fill volume achieved in semi-spherical and polyhedron containers was 87.7 \pm 2.2 mL and 71.1 \pm 3.3 mL respectively.

3.7.1.2 Sealing

The sealing of the containers was carried out in sanitized environment. For this phase of the testing, it was carried out in room environment with air in the headspace. The sealing of the containers was carried out in-house using a hydraulic pressure blister heat sealer. The conditions applied were 275 °F (135 °C) for 10 seconds at 100 psi (0.69 MPa). These conditions were found to be optimal in confirmation with ASTM D 3078 [77] in accordance to which a vacuum of 15 mm Hg (1999.8 Pa) was applied in dye leak detector equipment for 2 minutes.

The containers filled with the product were placed in a fitting cavity. Cushioning tape was applied at the edges of the cavity, so as to provide padding for good sealing. The lid was placed on top of the container and sealed. The containers and the lids were sanitized just before filling and sealing using Sodium Hypochlorite sanitizing solution. It was made sure that the sanitizing solution dried before filling. After sealing the containers and letting the seal cool down, the containers were checked visually for possible leakages by inverting the container and shaking the product inside the container.

3.7.1.3 Package Storage

After sealing, the containers were placed on corrugated partitions prepared for the purpose and placed under controlled conditions. For the first phase of testing, the containers were kept under room environment at 23 °C and 50% RH. Packages were stored in these conditions for 2 days and 7 days.

3.7.1.4 Quantification of Benzaldehyde

High Performance Liquid Chromatographic (HPLC) technique was used to quantify benzaldehyde in the drink system. Waters 2695 HPLC system with dual channel

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UV detector (model 2487) Nova-pak[®] C18 column (35 mm x 150 mm x 0.4 μ m) manufactured by Waters Corporation, Milford, Massachusetts was used. HPLC grade water and acetonitrile were used as the solvents in ratio of 65: 35. Table 3 lists the flow gradient used:

Start time	End time	Flow rate and condition
0 min	0.5 min	0 mL/ min- 0.1 mL/ min, gradient
0.5 min	2.5 min	0.1 mL/ min, isocratic
2.5 min	3 min	0.1 mL/min- 0.25 mL/ min, gradient
3 min	7 min	0.25 mL/min, isocratic
7 min	9 min	0.25 mL/ min- 1 mL/min, gradient
9 min	12 min	1 mL/min, isocratic
12 min	15 min	1 mL/ min- 0 mL/min, gradient

Table 3 Solvent flow gradient to determine benzaldehyde concentration

The injection volume was 5 μ L and the injection run time was 15 mins. Benzaldehyde eluted at around 9.5 mins. Each vial was used for 3 injections. At the end of each storage stage, samples were collected from 2 or more containers for analysis. Calibration curve was prepared using the following concentrations of benzaldehyde in methanol: 10.45, 41.8, 83.6, 167.2 ppm. The calibration curve is shown in the appendix J.

3.7.1.5 Sensory Testing with Consumer Panel

First stage of testing was carried out with consumer panel. This was used as a precursor to the final sensory testing and to make appropriate corrections. The study involved 40 panelists. This sensory test was conducted after 2 days and 7 days of storage of the product as a precursor for more intensive tests to follow. Testing was carried out in a standard triangle test format with no replication because of intense flavor of the product. The test was run on SIMS[®] 2000 software provided by Sensory Computer Systems, Morristown, NJ. The samples were randomized and presented in a balanced incomplete block (BIB) design to the panelists. Each panelist was presented with three

samples out of which two were from one type of container and one was from other type of container. Consumers were asked to pick the odd sample out. They had to guess, if they could not perceive any difference. Questionnaire for sensory testing is attached in the appendix F.2.

3.7.2 Phase II: Final Testing

3.7.2.1 Selection, Threshold and Training of Panelists

Based on the experience from the previous stage of testing, some corrections were made in the overall methodology. It was decided that a trained panel needs to be developed to be absolutely sure that the panelists are actually judging the benzaldehyde strength in the product and nothing else. The panelists were selected to comply with ASTM E 460-04 [78] which states that panelists should be screened in order to test for sensory effects of packaging on the product. The panelists were selected for further training. Rating test was used to select 15 panelists out of 37 panelists who participated in the screening test. Panelists were presented with 2 reference samples and 5 unidentified samples with different concentration of benzaldehyde, and they were asked to rate the samples on a scale of 0-7. Reference samples represented 0 and 7 on that scale. At the start of training of the trained panel, threshold study was conducted in confirmation with ASTM E679-04 [76] to find out the threshold of trained panel towards benzaldehyde in that particular drink system. The threshold of the panel was found to be 6.43 ppm. The panelists were trained for 5 weeks with varying concentrations of benzaldehyde and varying concentrations of citric acid and sugar that can affect perception of benzaldehyde. Only after satisfactory improvement in the performance of the panel as measured by the consistency and the proximity of perception in the group was observed, actual testing was started. The panelist performance curves showing performance enhancement are attached in appendix K.

3.7.2.2 Filling

The filling was carried out in the same way as for the pre-run testing in sanitized environment.

3.7.2.3 Sealing

The sealing of the containers was carried out in sanitized environment. For this phase of testing, it was carried out in nitrogenous environment. This allowed for the purging of headspace O_2 . Benzaldehyde is readily oxidized, removal of oxygen helped to avoid any risk of benzaldehyde being oxidized. Besides the sealing environment, all other parameters were exactly the same as with phase I testing.

3.7.2.4 Package Storage

After sealing, the containers were placed on corrugated partitions prepared for the purpose and placed under controlled conditions. For this phase of testing, the containers were kept under nitrogenous environment at 23 °C to avoid possible oxidation of benzaldehyde. The enclosing chamber had a volume of approximately 21400 in³ (350,683 cc). The chamber was flushed with medical grade nitrogen at least once in 3 days with fresh nitrogen to remove permeated benzaldehyde and possibly diffused air. Nitrogen stream was turned on every time when the chamber was opened to remove containers for testing. Also, positive pressure was maintained inside the chamber. The containers with product were stored in this condition for 3, 7, 14, 21 and 28 days.

3.7.2.5 Quantification of Benzaldehyde

Benzaldehyde quantification was carried out in exactly the same way as in Phase I testing presented in section 3.7.2.4.

3.7.2.6 Sensory Testing with Trained Panel

Out of 15 panelists selected after screening, only 9 were used for actual testing for logistical reasons. At least 8 of the panelists were present at each stage of the testing. The panelists were presented with one sample from each type of container and a control sample that did not have any benzaldehyde in it. Control sample was used as a reference sample and the panelists were asked to rate the other two samples on a scale of 0-7, with 7 being strongest and representative of a benzaldehyde concentration approximately 50 ppm. The questionnaire is attached in the appendix F.3. They were allowed to rate the samples equally if they perceived no difference in the samples.

3.8 Container Properties

3.8.1 Thickness Profile

Thickness of the containers was measured at 5 different spots on each container. From each type, 5 containers were tested. Magnamike 8500 thickness tester manufactured by Panametrics- NDT and provided by Olympus NDT Inc., Waltham, MA. The probe was first calibrated with manufacturer's instructions and steel ball size used was 1/8".

3.8.2 Water Vapor Transmission Rate

Water Vapor Transmission Rates (WVTR) of the containers were measured in accordance to ASTM F 1249-06 [79] using a MoCon Permatran[®] 3/33 manufactured by

Modern Controls, Inc., Minneapolis, MN at 37.8 °C and 100% RH. Three samples of each of the two types of containers were tested and the results were compared statistically. The containers were sealed on a metal platform using Loctite Extreme Repair adhesive manufactured by Henkel Corporation, OH. Metal platform was connected to Permatran system with copper tubing to carry N₂ into the container and back to the detector. This system was sealed in a polyethylene pouch along with wet sponges used to produce 100% RH inside. The test was continued until at least 10 steady state points (<5% WVTR variation) were achieved and the average of those last 10 points were taken to calculate the steady state permeation rate. Figure 8 explains the test set-up for WVTR testing.



Figure 8 Water Vapor Transmission Rate test setup

3.8.3 Oxygen Transmission Rate

Oxygen Transmission Rate (OTR) of the containers was determined in according to ASTM D 3985-05 [80] using a Illinois Instruments 8000 series oxygen analyzer, manufactured by Illinois Instruments, Johnsburg, IL. Three containers of each type were tested at 23 °C and 0% RH. The containers were sealed on a metal platform with Loctite Extreme Repair Adhesive provided by Henkel Corporation, OH. The metal platform was connected to the oxygen analyzer with copper tubing carrying N₂ in and out of the containers. The containers were tested with air as the permeant gas and the results were compensated to 100% oxygen. The test was continued until at least 10 steady state points (<5% OTR variation) were achieved and the average of those last 10 points were used to calculate the steady state permeation rate. Figure 9 explains the test set-up for OTR testing.



Room air as permeant

Figure 9 Oxygen Transmission Rate test setup

3.8.4 Crystallinity

Crystallinity of the containers was determined using TA Instruments Q100 Differential Scanning Calorimeter manufactured by TA instruments, New Castle, DE. The temperature calibration of equipment was performed in accordance with ASTM E 967-03 [81] and the heat flow calibration was performed in accordance with ASTM E 968-02 [82]. The enthalpies of fusion and crystallinity were measured and calculated in accordance with ASTM D 3418-03 [83]. Three containers of each type were tested. For polyhedron containers, four samples were extracted from the walls and two samples were extracted from the corners of the each polyhedron container. Four samples were cut from each semi-spherical container. All samples weighed between 5-8 mg. Only one heating cycle was used to measure the crystallinity. Samples were heated from 40 °C- 180 °C at a rate of 10 °C/min.

3.9 Statistical Techniques

Statistical techniques were applied to design the experiments and later on analyze the results. A type I error rate (α) of 5% was applied to all the experiments. For the phase I sensory test involving consumer panel, number of consumers (40) were calculated based on type I error rate (α) of 5%, power of test (1- β) of 80% and assumed proportion of distinguishers (p_d) of 30% [84]. To compare the variance and mean values of WVTR, OTR and BATR of containers F-test and t-Test were applied respectively. F-test and t-Test were also utilized to compare the ratings of the product from the two types of containers as adjudged by the trained panelists. Tukey's correction was applied to t-tests when comparing crystallinity in different parts of the containers. The type I error rate was divided by the number of possible comparisons and p-value was compared to this modified type I error rate. The statistical tests were conducted using Microsoft Excel[®] provided by Microsoft Corporation, Redmond, WA.

Chapter 4

Results

The results are presented in the following sections with container properties coming first and shelf life studies coming after that. Finally, discussion is presented to suggest the correlation between various factors and other possible causes of the results and variability if any. Statistical inference is included with all the results with a type I error rate of 5%.

4.1 Container Properties

4.1.1 Thickness

Thickness distribution of the two containers is profiled in figure 10 and figure 11.



Figure 10 Thickness distribution of semispherical container $*1\mbox{ mi}=25.4\mbox{ }\mu\mbox{m}$



The overall thickness of semispherical containers was 10.7 ± 3 mil while that of polyhedron containers was 13 ± 2.5 mil. Thickness of polyhedron containers was statistically higher than that of semispherical containers at a type I error rate of 0.05.

4.1.2 Water Vapor Transmission Rate (WVTR)

The WVTR of semi-spherical and polyhedron containers is shown in Figure 12





WVTR of the semi-spherical container was 0.017 ±0.001 gms/ pkg-day and that of polyhedron containers was 0.021±0.002 gms/ pkg-day at 37.8 °C and 100% RH. The WVTR of semi-spherical containers was 21% less than polyhedron containers. It is visible that both polypropylene containers have very low water vapor transmission rate but even at such low permeation rates, the differences between the two containers were statistically different. One implication of the low permeation rates is that at 23 °C and 50% RH (actual storage conditions for shelf life studies), the water loss from the simulated drink would be quite low. This will have very small or negligible effect on changing the overall flavor balance of the product during the storage. Thus, the results in shelf life studies are affected in a very limited manner because of the difference in WVTR. But the above results show that geometry affects the WVTR in a significant manner and for the packages with higher permeation rates, this can play critical role. The moisture sensitiveness of the product would also be a factor to consider in such scenario.

4.1.3 Oxygen Transmission Rate (OTR)

Figure 13 shows the OTR values for semispherical and polyhedron containers.





OTR measured with air as permeant was compensated to 100% oxygen concentration. OTR of polyhedron containers compensated to 100% oxygen was found to be 6.81±1.18 cc/ pkg-day and that of semi-spherical containers was 4.22±0.52 cc/ pkgday at 23 °C. Thus, the OTR of semi-spherical containers was 60% less than that of polyhedron containers. These results demonstrate that the geometry of the container affects the oxygen transmission rate significantly. Benzaldehyde is known to readily oxidize at room temperature. Since the product was stored in nitrogenous environment for the phase II studies, these effects were minimized for this phase. However, there is a possibility that this could have affected the phase I study, both of which are explained later. Since, the oxygen permeability of containers is affected by the geometry, it becomes essential to understand the phenomenon in order to improve the barrier. In commercial environment, products are usually not stored under nitrogen, and besides the flavor compounds, other components of food systems like lipids and vitamins also tend to oxidize.

4.1.4 Crystallinity

In earlier studies it has been shown that the extent of crystallinity affects the barrier properties of the films against organic compounds[4]. Thus, to see the difference in crystallinity, DSC tests were run on the polymer extracted from different locations in each container. Crystallinity of polypropylene in semi-spherical containers was found to be $30.7\pm1.1\%$. Crystallinity in walls of polyhedron containers was $30.3\pm0.5\%$ and in the corners of the polyhedron containers $29.3\pm0.9\%$. A graphical comparison is shown in the figure 14.





There is a statistically significant difference in the average values of crystallinity in walls of polyhedron containers and the polyhedron corners. Semi-spherical container walls are not statistically different as compared to corners or walls of polyhedron containers with a type I error rate of 0.05. Thus, it is difficult to say that crystallinity is playing a role for the difference found in the barrier properties of the two types of containers. It is suggested that two other factors could have played a synergistic role in determining the difference in the barrier properties. Differential orientation possibly caused by difference in geometries can change the barrier properties [13, 85]. The other factor of significance might be the distribution of crystallites. Drifting and realignment of crystals due to variable stresses caused can make the permeant path relatively more tortuous, ultimately affecting the barrier properties of the package. This study did not include measurement of these properties but these might be important avenues to look into for future studies to enhance understanding of the changes caused by the differential geometry.

4.2 Shelf Life Studies

Sensory and HPLC studies could establish that the differences in barrier properties discussed earlier might have a significant effect on the perceptibility of the cherry flavor by the consumers, if the differences are large enough to be measurable both by passive and human instruments. The above mentioned measured and suggested variables were affecting the flavor of the product as much as to bring it in the spectrum of practical significance.

4.2.1 Sensory Results

4.2.1.1 Consumer Panel

Consumer panel was used as a precursor for the final testing. At this stage, the product was sealed and stored under room conditions at 23 °C and 50% RH. Tested with triangle test, the consumers could see the difference in the products stored in two different types of containers as soon as 7 days. But it could not lead to a conclusive result because these results could not be verified with HPLC analysis which showed no presence of benzaldehyde in the product. This led to believe that the consumers are seeing difference in a different constituent of product because all of the benzaldehyde had probably oxidized completely or permeated completely. Though it did not undermine the fact that the two types of containers were different in terms of barrier properties, but with this, it can not be concluded that their barrier properties against the permeation of benzaldehyde were different. As discussed earlier the Oxygen transmission rates of the
two containers were different and that might have played a role because the containers were stored in the room environment at this stage and not in nitrogenous environment. The learnings taken from this stage were to develop a trained panel that would test only for the differences in benzaldehyde presence and to store the product in a san-oxygen environment

4.2.1.2 Trained Panel

Presented here are the results obtained with the trained panel that was utilized after making appropriate corrections to the overall methodology and training the panel. Appendix K presents the performance improvement of the trained panel in the 5 weeks of training. Figure 15 shows the average rating by the trained panel to the products stored in the two types of containers. A rating of 0 represents no perceptible benzaldehyde presence in the product and a rating of 7 corresponds to a concentration of 45 ppm or higher.



Figure 15 Benzaldehyde concentration rating

*Different letters over the dots indicate statistically significant difference at α=0.05 (o) semispherical, (*) polyhedron. [§]Average rating for five panelists from two containers.

The panel could detect differences significantly after day 21 and results were verified with day 28 samples. The average ratings for the products from the semispherical and polyhedron containers were 5 ± 0.13 and 3.38 ± 0.13 at the end of 21 days and 4.75 ± 0.11 and 2 ± 0.07 at the end of 28 days, respectively.

4.2.2 Benzaldehyde Concentration Determination

During the testing with the trained panel, the product was simultaneously tested with HPLC, whose results are presented here. Figure 16 shows the residual concentration of benzaldehyde in the product stored in the two types of containers after different time storages.





The benzaldehyde strength was found to be significantly more in product stored in semispherical containers than in polyhedron containers at the end of 21 and 28 days. The benzaldehyde concentration in semispherical containers was 40.39 ± 0.34 ppm and 38.84 ± 0.76 at the end of 21 and 28 days respectively while it was 19.06 ± 9.95 and 0.05 ± 0.025 in the polyhedron containers.

As shown in Figure 17, the sensory panel results and benzaldehyde concentration measured with HPLC technique are in good agreement with each other and follow the same trend.



Figure 17 Benzaldehyde panel rating and concentration measured by HPLC (▲) semispherical concentration, (▲) polyhedron concentration, (■) semispherical rating, (■) polyhedron rating

This confirms that the two tools devised to study benzaldehyde presence in the drink were individually correct and collectively veritable. One deviation observed was on day 14. On day 14, it was found that product from polyhedron container was rated higher than semispherical container. But in contrast to the ratings given by panelists, benzaldehyde strength in the products from two containers was not statistically different, though the trend seemed to follow. Thus, this difference in the ratings can be considered an aberration caused by external reasons. But it could not be repeated, and it was considered an aberration caused by outliers.

This study confirms that there is a relationship between package configuration and barrier properties of containers causing difference in the sensory flavor perception. This goes on to demonstrate that with application of design principles in appropriate manner, the barrier properties can be customized and the shelf life can be improved whenever mass transfer is concerned.

Chapter 5

Conclusions

5.1 Outcomes from the study

Restating the null and alternate hypothesis

Two polymeric containers made with same material and same surface area but in different shapes have the same barrier properties and the product stored will have the same sensory flavor perception.

Ha:

Ho:

Two polymeric containers made with same material and same surface area but in different shapes have different barrier properties and the product stored will have different sensory flavor perception.

This work established that the null hypothesis can be safely rejected with a type I error rate of 0.05. Thus, the shape of the container affects its barrier properties enough so as to cause difference in sensory flavor perception by the consumer.

It was demonstrated that the water vapor transmission rates and oxygen transmission rates are significantly different for the two differently shaped containers with a type I error rate of 0.05. Average WVTR of semi-spherical containers was 20% less than that of polyhedron containers, and average OTR of semi-spherical containers was 60% less than that of polyhedron containers.

The crystallinity in the walls of polyhedron container were found to be statistically different than the crystallinity in the corners of the container. The

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crystallinity in the semispherical container walls was different than the crystallinity in the corners of polyhedron container. Though the difference was statistically significant, in absolute terms, the walls of both semispherical and polyhedron containers did not have a considerable difference in crystallinity in comparison to crystallinity in the corners of the polyhedron container. In this scenario, the difference in barrier properties may be attributed to the possible difference of orientation in the two shapes and the distribution of crystallites.

The difference in the barrier properties of the two containers against benzaldehyde was found to be significantly affecting the sensory perception of the product stored in as soon as 21 days. The residual benzaldehyde in the polyhedron containers was less than that in the semi-spherical container as established by HPLC analysis of the product. Thus, sensory and HPLC results were in agreement establishing that the barrier properties are significantly different to affect the sensory perception of the product stored.

5.2 **Recommendations for Future work**

Though this work established that the container shape affects the barrier properties significantly, there is still scope of further work in this direction. Based on the limitations and results of this work, a need to follow up this study is recognized in following areas:

1. Orientation, distribution of crystallites and many other factors that define the morphology of a polymer need to be studied in such differently shaped containers as used in this study in order to precisely determine the reasons of differences in the barrier performance.

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2. A similar study can be conducted with other kinds of processing like injection molded and blow molded components. This will help understand if the specific processes bring more differences in the shapes and other processes do not. In such a case, these particular processes would need to be given special attention in a commercial production facility.

3. Another avenue for further research would be with other kinds of higher and lower barrier materials than PP and with other kinds of flavor compounds, individually and a mixture of the flavor compounds. This will help in demonstrating if this phenomenon is limited to particular polymer, permeant pairs or if the phenomenon is more general. When using more than one flavor compound, such a study will relate better to the commercial situation where the product usually have many flavor compounds. **Appendices**

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Appendix A Approval from Institutional Review Board (IRB)

Pankaj Kumar

From:	"IRB" <irb@ora.msu.edu></irb@ora.msu.edu>
Date:	Thursday, July 05, 2007 10:57 AM
To:	"Pankaj Kumar" <kumarpan@msu.edu></kumarpan@msu.edu>
Subject:	RE: About IRB# X05-337

Dear Pankaj Kumar,

Thank you for your inquiry regarding your project titled "Correlation between Sensory Aroma Perception and Package System Configurations." Our records indicate you have been listed as an additional investigator since this projects inception. Please give this correspondence to the graduate office. If this does not suffice and you need an official letter please let me know.

Thank you.

Judy McMillan IRB Staff Human Research Protection Programs Biomedical and Health Institutional Review Board (BIRB) Community Research Institutional Review Board (CRIRB) Social Science/Behavioral/Education Institutional Review Board (SIRB) Office of Regulatory Affairs 202 Olds Hall Michigan State University

East Lansing, MI 48824-1046 Phone: (517) 355-2180 Fax: (517) 432-4503 Email: irb@msu.edu

Website: www.humanresearch.msu.edu

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Initial IRB Application Approval

April 14, 2005

To:	Rafael Auras
	140 Packaging

Re:	IRB # 05-337	Category: EXEMPT 1-6
	Approval Date:	April 14, 2005
	Expiration Dete:	April 13, 2006

Tille: CORRELATION BETWEEN SENSORY AROMA PERCEPTION AND PACKAGE SYSTEM CONFIGURATIONS

The University Committee on Research Involving Human Subjects (UCRIHS) has completed their review of your project. I am pleased to advise you that your project has been approved.

The committee has found that your research project is appropriate in design, protects the rights and welfare of human subjects, and meets the requirements of MSU's Federal Wide Assurance and the Federal Guidelines (45 CFR 46 and 21 CFR Part 50). The protection of human subjects in research is a partnership between the IRB and the investigators. We look forward to working with you as we both fulfill our responsibilities.

Renewals: UCRIHS approval is valid until the expiration date listed above. If you are continuing your project, you must submit an *Application for Renewal* application at least one month before expiration. If the project is completed, please submit an *Application for Permanent Closure*.

Revisions: UCRIHS must review any changes in the project, prior to initiation of the change. Please submit an Application for Revision to have your changes reviewed. If changes are made at the time of renewal, please include an Application for Revision with the renewal application.

Problems: If issues should arise during the conduct of the research, such as unenticipated problems, adverse events, or any problem that may increase the risk to the human subjects, notify UCRIHS promptly. Forms are available to report these issues.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with UCRIHS.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at <u>UCRIHS@msu.edu</u>. Thank you for your cooperation.

Sincerely,

Polas-

Peter Vasilenko, Ph.D. UCRIHS Chair

c: Janice HARTE 114 Trout FSHN Bidg.

School of Packaging, Michigan State University

Consumer Panel Consent Form

Evaluation of Packaging Cherry/Fruit Drink Applications

Dear Participant:

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Michigan State University researchers are investigating packaged fruit/cherry flavored drinks and asking that panelists participate in a storage study of packaged cherry drink stored in plastic packages (Poly Propylene). We are asking for volunteers, over the age of 18, taste these samples. If you have a known food allergy to any of the following possible FDA approved food ingredients: sugar, citric acid and artificial fruit flavors, stabilizers/guns please do not volunteer for this study. Packaging materials are approved by the FDA for contact with food. Each evaluation will take about 10 minutes after you receive your samples. You will be given a coupon or food treat worth \$2 or less as an appreciation for your participation and completion of the questionnaires.

Though none is anticipated, if you have a problem **upon sniffing and tasting the samples**, notify the onsite sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have. Your response is confidential and we will protect your confidentiality to the full extent of the law.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principal investigator, Dr. Rafael Auras, via phone at 517-432-3254, or regular mail at 130 Packaging Building, East Lansing, MI 48824. He can also be reached via email at <u>aurasraf@msu.edu</u> for any inquiry you might have related to your participation in the study. In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Michigan State University's Chair of University Committee on Research Involving Human Subject (UCRIHS) by phone: (517) 355-2180, fax: (517) 432-4503, email: <u>ucrihs@msu.edu</u> or regular mail: 202 Olds Hall, East Lansing, MI 48824.

I voluntarily agree to participate in the study.

SIGNED _____

DATE

UCRIHS APPROVAL FOR THIS project EXPIRES:

APR 1 8 2006

SUBMIT RENEWAL APPLICATION ONE MONTH PRIOR TO ABOVE DATE TO CONTINUE

MICHIGAN STATE

April 6, 2006

To: Rafael Auras 140 Packaging

Renowal Application Approval *Excerned

Re: IRB # X05-337 Category: Exempt Approval Date: March 29, 2006

Title: Correlation between Sensory Aroma Perception and Package System Configurations

The Biomedical and Heath Institutional Review Board (BIRB) has completed their review of your renewal application. I am pleased to advise you that your project has been confirmed as exempt and approved under our new exempt policy.

The IRB has found that your research project meets the criteria for exempt status and the criteria for the protection of human subjects in exempt research. Under our exempt policy the **Principal Investigator assumes the responsibilities for the protection of human subjects in** this project as outlined in the assurance letter and exempt educational material. The office has received your signed assurance for exempt research. A copy of this signed agreement is appended for your information and records.

Renewals: Exempt protocols do <u>not</u> need to be renewed. If the project is completed, please submit an *Application for Permanent Closure*.

Revisions: Exempt protocols do <u>not</u> require revisions. However, if changes are made to a protocol that may no longer meet the exempt criteria, a new initial application will be required.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects and change the category of review, notify BIRB promptly. Any complaints from participants regarding the risk and benefits of the project must be reported to the BIRB.

Follow-up: If your exempt project is not completed and closed after <u>three years</u>, BIRB will contact you regarding the status of the project and to verify that no changes have occurred that may affect exempt status.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with UCRIHS.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at <u>irb@msu.edu</u>. Thank you for your cooperation.

Sincerely,

Peter Vasilenko, Ph.D. Biomedical and Health Institutional Review Board Chair

C: Janice Harte 114 Trout FISN Bldg.

Principal Investigator Assurance of An Exempt Project

Name of Principal Investigator: Rafael Auras

Bich

MAR 3 0 2005

Title of Project: Correlation between Sensory Aroma Perception and Package System Configurations

IRB #: X05-377 ×05-337

The University Committee on Research Involving Human Subjects (UCRIHS) has deemed this project as exempt, in accord with the federal regulations for the protection of human subjects. As an exempt project, the IRB will not be further involved with the review or continued review of the project, as long as the project maintains the properties that make it exempt.

- Since the IRB is no longer involved in the review and continued review of this project, it is the Principal Investigator who assumes the responsibilities for the protection of human subjects in this project and ensures that the project is performed with integrity and within accepted ethical standards; particularly as outlined by the Belmont Report (see exempt educational materials).
- The Principal Investigator assumes the responsibility for ensuring that research subjects be informed of the research through a documented or undocumented consent process, if appropriate.
- The Principal Investigator assumes the responsibility to maintain confidentiality of the subjects and the data, and maintain the privacy of the subjects and protection of the data through appropriate means. If data is anonymous, the investigators will make no attempt to identify any individuals.
- The Principal Investigator assumes responsibility for assuring that human subjects will be selected equitably, so that risks and benefits of research are justly distributed.
- The Principal Investigator assumes the responsibility that co-investigators and other members of the
 research team adhere to the appropriate policies for the protection of human subjects, maintain
 confidentiality and privacy, and adhere to accepted ethical standards.
- If the Principal Investigator adds additional investigators to an exempt project, he/she may inform UCRIHS
 of the additions. This may be of particular importance to graduate students if the MSU Graduate School
 requires proof of IRB approval.
- Any complaints from participants regarding the risks and benefits of the project must be reported to UCRIHS. Any information, unexpected or adverse events that would increase the risk to human subjects and cause the category of review to be upgraded to Expedited or Full Review must also be reported to UCRIHS.
- Since the Principal Investigator and co-investigators are charged with human subject protection and
 adhering to ethical principles in exempt research, it is appropriate that investigators be trained in human
 subject principles. The Principal Investigator and all members of the research team are required to
 complete MSU IRB educational requirements or equivalent.
- Any change in the project that may raise the project from exempt to an expedited or full review category
 must be presented to UCRIHS. If there is any question about a change in the project, the Principal
 Investigator should consult the Chair of UCRIHS. Failure to submit changes that raise the project out of
 the exempt category will be considered non-compliance and will be subject to investigation and action by
 UCRIHS.

By signing below, the Principal Investigator assures that he/she will abide by the terms of this assurance and the UCRIHS exempt policy.

Signature of Principal Investigator

03/29/0C Date

6/27/05

B.1 Drink composition



Figure B-i Process Flow for drink composition

B.2 Making containers



Figure B-ii Process Flow for making containers

Appendix C Stability testing of drink

The product was tested under N_2 and O_2 for stability and presence of benzaldehyde. Product was stored under air and N₂ headspace. For this purpose 60 ml glass bottles were utilized and 6 ml of product was stored in these bottles. Half of the bottles with each type of headspace were exposed to light and the other half were covered with foil to protect from light. The samples were stored at room temperature. Comparison between air and N₂ headspace was carried out up to 4 days. The samples were tested with HPLC technique described in the main body of the thesis. Starting concentration was 46.63 ± 0.45 ppm. The concentrations of benzaldehyde with air in headspace in dark and light exposed conditions at the end of 4 days were 41.35 ± 0.07 ppm and 41.10 ± 0.09 ppm respectively. The concentration at same time with N_2 in headspace and dark and light exposed conditions were 39.54 ± 0.51 and 36.77 ppm, respectively. This suggests that there was not a considerable difference in the product stability with N_2 or air headspace since the degradation rate was not significant. It was also observed that the initially the concentration went down faster, most probably owing to the evaporation of benzaldehyde and then it became stagnant. To assess the effects of light further with N₂ in headspace, the experiment was repeated with only N₂ headspace for up to 21 days. At the end of 18 days, the concentrations in the dark and light exposed conditions were 15.68 ± 6.46 and 8.82 ± 7.3 ppm, respectively. At the end of 21 days, these concentrations were 20.24 \pm 8.5 and 9.02 \pm 0.96 ppm. No definitive effect could be assessed on benzaldehyde concentration because of presence or absence of light with these results.

Based on these experiments, where huge headspace was provided to the product, and based on the concentrations seen after day 18 and day 21, it can be said that the product is shelf stable at room conditions under both N₂ and air headspace. In these experiments, detection wavelengths used were 201 nm and 265 nm, and at these wavelengths, most of the compounds can be seen. No additional peak was detected at any of the stages, suggesting that all the loss of benzaldehyde was entirely due to evaporation and not due to degradation. Evaporation of benzaldehyde was slow and thus, attaining equilibrium between headspace and dissolved benzaldehyde was slow too. This is confirmed when partial pressure of benzaldehyde is calculated and compared using Antoine equation and ideal gas law at 23 °C. Partial pressure calculated from Antoine equation (P_a) was 11.5 Pa. The ideal gas equation yielded a partial pressure (P_i) of 2.43 x 10^7 Pa. As is apparent that P_i>>>P_a, the evaporation of benzaldehyde would keep on taking place for very long time since the driving force is available. This also means that equilibrium between headspace and liquid phase will take a long time to achieve. Appendix D Sensory promotional flyer

Participate in a quality evaluation of Packaged Fruit/Cherry Drink!



We are seeking panelists to participate in a consumer research study that will involve evaluation of packaged cherry drink. Each evaluation will take about 10 minutes or less.

where?102 Trout FSHN, MSUWhen?March 16, 20079 AM-1 PMWhat?Packaged Fruit/cherry Drink packaged in FDAapproved packages

Food treat coupons will be provided following tasting the drink and completion of the survey.

Any questions? Contact (517-432-3254), Dr. Rafael Auras in the School of Packaging, <u>aurasraf@msu.edu</u>

Appendix E Consent Forms

E.1 Consumer Panel Consent form

Evaluation of Packaging Configuration for Packaged Cherry Drink Applications

Dear Participant:

Michigan State University researchers are investigating **packaged cherry drink** and asking that panelists participate in a storage study of packaged cherry drink stored in plastic packages (Poly Propylene). These materials are approved by the FDA for contact with food. Each evaluation will take about 10 minutes or less after you receive your samples. You will be given a coupon or food treat worth \$2 or less as an appreciation for your participation and completion of the questionnaires.

Though none is anticipated, if you have a problem **upon sniffing and tasting the samples**, notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have. Your response is confidential and we will protect your confidentiality to the full extent of the law.

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If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator, Dr. Rafael Auras, via phone at 517-432-3254, or regular mail at 130 Packaging Building, East Lansing, MI 48824. He can also be reached via email at <u>aurasraf@msu.edu</u> for any inquiry you might have related to your participation in the study. In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Ph.D., Director of Human Research Protections, by phone: (517) 355-2180, fax: (517) 432-4503, email: <u>irb@msu.edu</u> or regular mail: 202 Olds Hall, East Lansing, MI 48824-1047.

I voluntarily agree to participate in the study.

SIGNED _____ DATE_____

E.2 Trained Panel Consent Form

(Page 1)

Evaluation of Packaging Configuration for Packaged Cherry Drink Applications

Dear Participant:

Before you decide to sign this consent form and continue to participate in this study, please read this document carefully for the information related to the study, ingredients, packaging material and procedures used in the study. Potential risks and benefits from your study, assurance of your privacy and your rights as a human subject in our study are also listed.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator, Dr. Rafael Auras, via phone at 517-432-3254, or regular mail at 130 Packaging Building, East Lansing, MI 48824. He can also be reached via email at <u>aurasraf@msu.edu</u> for any inquiry you might have related to your participation in the study. In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Ph.D., Director of Human Research Protections, by phone: (517) 355-2180, fax: (517) 432-4503, email: <u>irb@msu.edu</u> or regular mail: 202 Olds Hall, East Lansing, MI 48824-1047.

PLEASE NOTE THAT UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN THIS STUDY. YOUR SIGNATURES INDICATE YOU HAVE READ ALL THE INFORMATION PROVIDED IN THIS CONSENT FORM AND THAT YOU HAVE HAD AN

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ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS CAN BE PROVIDED UPON YOUR REQUEST.

I voluntarily agree to participate in the study.

SIGNED _____ DATE_____

Trained Panel Consent Form continued (Page 2)

Invitation to Participate: You are invited to participate in the study that assesses the effects of container configurations on the flavor barrier properties of packages for a simulated cherry drink.

Purpose of the study: We are investigating **packaged cherry drink** and asking that panelists participate in a storage study of packaged cherry drink stored in plastic packages (Poly Propylene). This study would help establish if the container shape affects the barrier properties of the package in preserving the flavor of cherry drink.

Procedure of the study: Each panelist would be served simulated cherry drink stored in different containers and would be asked to rank the sample after tasting it. Each sample would be coded with a unique 3-digit code. We are asking that panelists participate in this study that will be conducted over a 2-month period. Training will consist of approximately 3-4 sessions of 30-60 minutes. After training, approximately 6 evaluations will be scheduled. Evaluations should last about 30 minutes or less.

Sample Preparation: All the materials used in this study are approved by the FDA for contact with food. The product would be stored in the containers made with food grade plastic (polypropylene) at ambient conditions. Following is the composition of the product:

Water, Sugar, Citric Acid (for tartness), Vitamin E, Gum, Benzaldehyde (Cherry flavor)

Potential Risks: Since all the ingredients are FDA approved food grade materials in this study, these samples pose no adverse health risk, provided the subject has not been identified as being susceptible to an allergic reaction to the previously listed sample ingredients. Though none is anticipated, if you have a problem upon sniffing and tasting the samples, notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have. Your response is confidential and we will protect your confidentiality to the full extent of the law.

Expected Benefits: This study would enable the researchers understand that the phenomenon of flavor barrier better in formed containers. This in turn will help the food packaging industry devise better strategy to preserve flavor of the product and elongate sensory shelf life at an optimum cost.

Assurance of confidentiality: Any information obtained in connection with this study that could be identified with you will be kept confidential by ensuring that all consent forms and response sheets are securely stored. All data collected and analyzed will be reported in an aggregate format that will not permit associating subjects with specific responses or findings. Your privacy will be protected to the maximum extent allowable by law.

Withdrawal from the study: Participation in this study is voluntary. Your decision to refuse participation or discontinue participation during this study will be honored promptly and unconditionally.

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Appendix F Sensory Questionnaires

F.1 Questionnaire for measuring sensory threshold of benzaldehyde

3-AFC Threshold Test			
Name		Date	
Sample: Package	ed Cherry Drink	#	<pre># panelist number</pre>
Instructions		<u> </u>	
Five independent	sets (A, B, C, D, E) of	samples will be presented	l sequentially.
Each set has 3 dif	fferent samples; out of	which two are same, deter	mine the odd one.
If no difference	is apparent, you must	guess.	
Smell and taste samples from left to right. You may spit the sample out after judging (foam cup is provided). Which one is the odd one? (Check the box)			
Set A	□ 328	481	□ 869
Set B	□ 298	□ 966	□ 543
Set C	□ 769	□ 849	□ 228
Set D	D 488	□ 125	□ 252

COMMENTS:

Set E

D 747

□ 885

□ 526

F.2 Questionnaire for consumer panel sensory testing

Triangle Test				
Name Date Sample: Packaged Cherry Drink # panelist number				
Instructions There are 3 different samples; out of which two are same, determine the odd one. If no difference is apparent, you must guess. Smell and taste samples from left to right. Rinse the pallet with crackers and/ or water between samples. You may spit the sample out after judging (foam cup is provided). Which one is the odd one? (Check the box)				
Sample	□ 385	□ 496	□ 647	

COMMENTS: _____

F.3 Trained panel rating questionnaire

Date:

Type of Sample: Cherry (Benzaldehyde) Flavor

Characteristic Studied: Strength of Cherry Flavor in various packages after storage

Instructions:

- 1. Receive the samples and note each sample code in the next section according to its position on the tray.
- 2. Taste the samples from left to right and note the degree of strength of cherry flavor. Wait at least 30 seconds or more as appropriate between samples and rinse palate as required.
- 3. The sample coded with the letter "C" is control sample without any concentration of benzaldehyde. It is for your reference. You do not have to rate that sample in this questionnaire.
- 4. Rate the samples according to the following key for the cherry flavor profile.

0 1	Imperceptible	2 3	Slightly perceptible
4 5	Moderately perceptible	6 7	Strongly Perceptible

Comments:

Concentration	Correct Responses
4.9 ppm	23
14.7 ppm	22
44.1 ppm	26
132.3 ppm	25
396.9 ppm	28

Appendix G Panelist Response for Benzaldehyde threshold study

Table G.1 Number of correct responses for different benzaldehyde concentrations

Individual Threshold was calculated by the finding geometric means of the highest concentration that the panelist evaluated incorrectly and the next higher concentration. If the panelist evaluated all samples correctly, then it was assumed that the concentration one step lower than the least concentration (4.9 ppm/3= 1.5 ppm) would yield an incorrect response and the geometric mean of 1.5 ppm and 4.9 ppm was taken as individual threshold value. If the panelist gave an incorrect response at the highest served concentration, then it was assumed that the next higher step (396.9 ppm x 3= 1190.7 ppm) would yield a correct response. In this case, the geometric mean of 396.9 ppm and 1190.7 ppm was treated as the individual threshold.

Group threshold was calculated by taking geometric mean of the individual threshold values. It was found that the Group threshold for benzaldehyde in this drink system was 15.25 ppm.

Appendix H Codes for creating designs in Rhinoceros[®] 3.0

H.1 Semispherical Mold

A rectangular block of 12.7 cm x 12.7 cm x 12.7 cm was drawn. A sphere was drawn with its center coinciding with the top surface of the rectangular block. The sphere was added to the rectangular block to provide the unified structural design for the semi-sphere mold.

Command: File> New Select "Centimeters" as the template. Command: Solid> Box> corner to corner, height Parameters: First corner of Base: 0,0,0 Other corner of Base: 12.7, 12.7, 0 Height: 5 Command: Solid> Sphere> Center, Radius Parameters: Center: 6.35, 6.35, 5 Radius: 3.63 Command: Solid> Union First click on rectangular block, hit enter,

H.2 Polyhedron Mold

A rectangular block of 12 cm x 12 cm x 5 cm was drawn using box tool. A hexagon was drawn with sides measuring 4.80 cm with the center coinciding with the center of top surface of the rectangular block. The hexagon was extruded with a draft angle of 45° and height of 3.03 cm to provide a bottom edge dimension of 1.30 cm to the

polyhedron. The polyhedron was added to the rectangular block to provide unified structural design for the polyhedron mold

Command: File> New Select "Centimeters" as the template. Command: Solid> Box> corner to corner, height Parameters: First corner of Base: 0,0,0 Other corner of Base: 12, 12, 0 Height: 5 Command: Curve> Polygon> Center, Radius Click on NumSide and enter 6 Click on circumscribed Parameters: Center: 6, 6, 5 Midpoint of Polygon Edge: 10.15, 6, 5 Command: Surface> Extrude> Tapered Click on Draft Angle, enter -45 and hit enter Enter 3.03 for Extrusion Distance and hit enter Command: Solid> Union First click on rectangular block, hit enter, click on polyhedron, hit enter

Appendix I Deskproto Milling Parameters

Milling Tool: 3/8" diameter flat tip milling tool

Operation Parameters:

General

a. Distance between tool paths= 0.00778"

b. Stepsize along tool paths= 0.00778"

c. Feed rate= 10 inch/ min

d. Spindle speed= 1000 rpm

- □ Strategy
 - a. Main= block

b. Detail Settings, block= inside out

□ Roughing

a. Roughing= use

b. Layer Height= Custom, 0.04"

c. Protect Vertical Surfaces

Segment

a. Use segment of part

□ Borders

a. Size of the borders= No extra

Movement

a. Milling direction= Conventional

b. Free movement height= 0.0250"

c. Feed rate for plunge movement= 100% of federate

d. Feed rate for high chip loads= 50% of feedrate

□ Advanced

a. Ambient skipping= skip total ambient

b. Vertical surfaces= Do not check for vertical surfaces

 \square Simulation

a. Level of detail= High

Note: Two major problems faced once the designs were created and processed with Deskproto[®]. The available version of CPmill[®] software (milling machine software) could only be used with Windows 95[®] only, and because of that there were unexpected problems. Every stroke performed by the machine was 4-times the expected stroke length as defined by the design and the parameters. The problem was resolved by scaling the drawing down to 1/4th of original size and defining all the parameters again as 1/4th of original. All of these corrections were carried out in Deskproto[®].

CPmill[®] runs on Windows[®] 95 where it could not execute more than 36,000 command lines when running a milling program. Windows[®] would give an error message after executing 36,000 lines and would close the software. For a workable milling operation with enough fineness, the command program had around 92,000 command lines. The problem was resolved by manually creating 3 parts of the command program. With this, it was required to bring back the mill tool to the original starting point called "home" at the start of each program.

Appendix J Calibration curve for benzaldehyde quantification

Figure J-i presents the calibration curve for quantification of benzaldehyde concentration in the product. The benzaldehyde was prepared in different concentrations in methanol and the area response was measured in the HPLC.



Figure J-i Benzaldehyde Calibration Curve for HPLC

Appendix K Panelist Performance Charts

Figure K-i shows the panelist performance in rating the benzaldehyde strength on a scale of 0-7 when presented with three different samples of varying strength. Various lines correspond to the different panelists. It is visible that the panelists were not on the same scale and their responses were not proportional or consistent to the intended scale.


Figure K-ii shows the performance of panelists in week when presented with two samples to rate. It is apparent the extent of improvement that panelists achieved with most of them rating the samples in same direction though with limited variability in rating the overall strength. Important development was that they were able to rate the samples consistently as was observed with Week 5 training as well.





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