Effect of Shelf-life and Light Exposure on Acetaldehyde Concentration in Milk Packaged in HDPE and PETE Bottles

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(ABSTRACT)

Poly(ethylene terephthalate) (PETE) packaging is becoming an increasingly popular choice of packaging material for milk, but has the disadvantage of releasing odorous acetaldehyde into food matrices.

Sensory detection group thresholds for acetaldehyde in whole, low fat and nonfat unflavored milks were 3939, 4020, and 4040 ppb respectively with no significant difference due to fat level. Chocolate flavored milk and spring water showed detection thresholds levels for acetaldehyde of 10048 and 167 ppb respectively. This information assisted in determining if acetaldehyde migration from the package to the product would influence the flavor of the product.

Whole milk was packaged in glass, high density polyethylene (HDPE), amber PETE, clear PETE, and clear PETE with UV light block and was exposed to fluorescent light of 1100-1300 lux (100-120 FC) at 4^{o}C for 18 days. Sensory and chemical analysis and was done on milk from all containers over a period of 18 days. Emphasis was on oxidation, acetaldehyde and lacks freshness off-flavors and byproducts.

All volatile flavor compounds studied (acetaldehyde, pentanal, dimethyl disulfide, and hexanal) were increased in light-exposed milk samples. Amber PETE showed the least amount of oxidation off-flavor, while clear PETE with UV block showed significantly less oxidation off-flavor than glass, clear PETE or HDPE on day 7 and 18. Acetaldehyde was not detected by sensory analysis in either light-exposed or light-protected samples. Chemical analysis showed relative acetaldehyde levels in glass (2220 ppb), HDPE (1265 ppb), amber PETE (3397 ppb), clear PETE (2930 ppb), and clear PETE with UV light block (1754 ppb) were all below concentrations found for human flavor threshold.

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TABLE OF CONTENTS

		Page
TITLE		*i*
ABSTRACT		*ii*
ACKNOWLEDGEMENTS		*iii*
TABLE OF CONTENTS		*iv*
LIST OF TABLES		*vi*
LIST OF FIGURES		*vii*
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	Human threshold for acetaldehyde	3
	Methods of analysis	4
	Implication of processing and storage on vitamin and volatile compound concentrations in milk	6
	Acetaldehyde migration from packaging to product	9
	Acetaldehyde as inhibitor against pathogens	12
	References	14
III.	HUMAN FLAVOR THRESHOLD FOR ACETALDEHYDE IN MILK OF VARIOUS FAT CONTENT, CHOCOLATE MILK, AND MINERAL WATER	18
	A. Abstract	18
	B. Introduction	19
	C. Materials and Methods	20
	D. Results and Discussion	25
	E. Conclusion	33
	F. Acknowledgement	34
	G. References	35

Page

IV. EFFECT OF SHELF-LIFE AND LIGHT EXPOSURE ON ACETALDEHYDE CONCENTRATION IN MILK PACKAGED IN HDPE AND PETE BOTTLES 37

A. Abstract 37

B. Introduction 39

C. Materials and Methods 41

D. Results and Discussion 45

E. Conclusion 57

F. Acknowledgements 59

G. References 60

V. APPENDICES 62

A. Chocolate Milk Formula 63

B. Dilution Chart for Spiking 64

C. Human Subjects Consent Form 65

D. Scorecard for Sensory Evaluation of Acetaldehyde Threshold 68

E. Sample Preparation for Training of Panelists 69

F. Scorecard for Sensory Evaluation of Off-flavor Determination 70

G. Bacterial Counts (log CFU/ml) for Various Containers 71

H. Human Threshold for Acetaldehyde in Different Mediums 73

LIST OF TABLES

Page

Chapter III

1. Threshold for acetaldehyde in unflavored milk (nonfat, low fat, and whole), chocolate flavored milk and spring water as determined by the geometric mean approach 26
2. Predicted concentration of acetaldehyde at probability of 66.7% using logistic regression as compared to group threshold using geometric means 28
3. Acetaldehyde recovery from whole milk (3.25 % milkfat) using solid phase micro-extraction concentration technique 31
4. Acetaldehyde recovery from spring water using solid phase micro extraction concentration technique 32

Chapter IV

1. Relative area ratios of hexanal in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block 48
2. Relative area ratios of dimethyl disulfide in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block 48
3. Relative area ratios of pentanal in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block 49
5. Relative area ratios of acetaldehyde in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block 56

LIST OF FIGURES

Page

Chapter III

1. Probability of correct identification of acetaldehyde spiked samples using logistic regression when $p(x) = 1 / [1 + \exp(- \alpha - \beta x)]$ 28
2. Acetaldehyde in milk medium (3.25% milkfat) at 200 ppb (a, acetaldehyde; b, acetone; c, n-butanone; and d, 4-methyl-2-pentanone (internal standard)) 30
3. Acetaldehyde in water medium at 20 ppb (a, acetaldehyde; and d, 4-methyl-2-pentanone (internal standard)) 30
4. Calibration curve for acetaldehyde in whole milk (3.25% milkfat) 32

Chapter IV

1. Oxidation off-flavor in milk packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block when (a) exposed to light and (b) protected against light (off-flavor intensity: 1 – not noticeable; 9 – very strong) 46
2. Light transmission spectra of clear PETE, amber PETE, clear PETE with UV light block, and HDPE 47
3. Volatile compounds in fresh milk (a, acetone; and b, n-butanone) 49
4. Volatile compounds in milk after 18 days of storage in glass, HDPE, amber PETE, clear PETE, clear PETE with UV light block when protected against light (a) retention times 1.2 – 2.2 min and (b) retention times 2.2 – 9.0 min (a, acetaldehyde; b, acetone; c, n-butanone; d, pentanal; e, dimethyl disulfide; and f, hexanal) 50
5. Volatile compounds in milk after 18 days of storage in glass, HDPE, amber PETE, clear PETE, clear PETE with UV light block when exposed to light (a) retention times 1.2 – 2.2 min and (b) retention times 2.2 – 9.0 min (a, acetaldehyde; b, acetone; c, n-butanone; d, pentanal; e, dimethyl disulfide; and f, hexanal) 51

Page

6. Lacks freshness off-flavor in milk packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block when (a) exposed to light and (b) protected against light (off-flavor intensity: 1 – not noticeable; 9 – very strong) 53

7. Acetaldehyde off-flavor in milk packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block when (a) exposed to light and (a) protected against light (off-flavor intensity: 1 – not noticeable; 9 – very strong) 55

CHAPTER I

INTRODUCTION

Shelf-life, temperature, exposure to ultra-violet (UV) light, and the migration of volatile components from certain plastic polymers used in packaging play an important role in off-flavor development in milk (Bassette and Jeon, 1984; Rerkrai *et al*., 1987). The rate of chemical reactions yielding unwanted volatile flavor products such as acetaldehyde, propanal, n-butanal, and n-hexanal, increase more rapidly in milk products stored at higher temperatures, exposure to light, and with time. This leads to flavor changes that can decrease acceptability of milk (Rerkrai *et al*., 1987; Grieg and Manning, 1983).

Acetaldehyde is present in a large number of natural food products in concentrations up to 1000 mg/L (vinegar) (Van Straten *et al*., 1983). In dairy products it is mostly identified with fermented products, especially yogurt (Bodyfelt *et al*., 1988). Low pasteurized (12 sec at 73^{o}C) fresh milk naturally contains acetaldehyde at 10 ppb (Maarse, 1991). This level increases with exposure to light as a result of the photoreduction of riboflavin and the photogeneration of superoxide anion. This will subsequently lead to oxidation of polyunsaturated fatty acids and resulting in the formation of numerous volatile carbonyl compounds (Cladman *et al*., 1998; Cadwallader and Howard, 1998; Jeng *et al*., 1988). Acetaldehyde is also present in the polymer poly(ethylene terephthalate) (PETE) as a thermal degradation product formed during the melt condensation reaction and melt processing of PETE (Nijssen *et al*., 1996).

The packaging of milk plays a big role in consumer acceptability, with the emphasis on convenience, visibility of the product, and ease of use. Milk is currently mostly packaged in translucent or opaque/colored high density polyethylene (HDPE) containers, which is a relatively cheap material. PETE, however, has several advantages over HDPE because of its considerable mechanical resistance, characteristics of lightness, transparency, and gas tightness (Poretta and Minuti, 1995). A concern about the use of PETE as packaging material is the possible migration of acetaldehyde from the package to the product, which can lead to acetaldehyde levels above

human flavor threshold. Few studies have been done on acetaldehyde threshold in milk. Bills *et al*. (1972) determined the threshold for acetaldehyde in whole milk as 800 ppb at 5^{o}C.

This study was conducted in two phases. During the first phase the flavor threshold of acetaldehyde in whole (3.25 % milkfat), lowfat (2 % milkfat), nonfat (0.5 % milkfat), chocolate milk (3.25 % milkfat), and spring water was determined. This information assisted in determining if acetaldehyde migration from the package to the product would influence the flavor of the product.

During the second phase, the effect of different packaging materials (high-density polyethylene (HDPE), standard clear PETE, amber PETE, clear PETE with UV light block, and glass (control)) on the flavor of milk was determined. This study concluded if PETE is as effective in preserving milk quality as HDPE. The effect of storage time and light exposure on the milk quality, with regards to acetaldehyde concentration, was also evaluated.

CHAPTER II

REVIEW OF LITERATURE

Human threshold for acetaldehyde

Acetaldehyde is the second simplest aldehyde (next to formaldehyde). It is a colorless liquid with a boiling point of 21^{o}C and therefore volatile at room temperature and pressure. Pure acetaldehyde possesses a pungent irritating odor but at dilute concentrations it gives a pleasant fruity aroma (Arctander, 1969). It is present in a large number of natural food products in concentrations up to 1000 mg/L (vinegar) (Van Straten *et al*., 1983).

In dairy products acetaldehyde is most often identified with fermented products, especially yogurt (5-40 mg/L) (Bodyfelt *et al*., 1988). The amount of acetaldehyde required for the development of a characteristic flavor in cultured dairy products varies widely according to the product. In natural yogurt, acetaldehyde is the chemical compound most responsible for providing the characteristic "natural style" or "green apple-like" flavor. Both *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Bodyfelt *et al*., 1988) readily produce it.

Milk does not contain acetaldehyde in large quantities. Maarse (1991) determined that low pasteurized (12 sec at 73^{o}C) fresh milk contains 10 ppb acetaldehyde. Bills *et al*. (1972) determined that the flavor thresholds for acetaldehyde in milk, milk with 8% sucrose, and milk with 8% sucrose and 0.4% strawberry flavor were 800, 1000 and 11700 ppb respectively as confirmed by gas-liquid chromatography, with gas entrainment on-column trapping technique and sampling at 5^{o}C. Flavor detection threshold can be defined as an energy level below which no sensation would be produced by a stimulus and above which a sensation would reach consciousness (Lawless and Heymann, 1998).

Since fat is the major source of flavor compounds in milk, including acetaldehyde, the flavor threshold for acetaldehyde in whole milk is expected to be higher than in skim milk (Miyake and Shibamoto, 1993; Yasuhata and Shibamoto, 1991). At comparable levels of acetaldehyde in

plain and strawberry flavored milk, acetaldehyde seemed much less pronounced in the flavored milk, due to masking of the acetaldehyde by the strawberry flavor (Bills *et al*., 1972).

In the mineral water industry it is generally assumed that the odor threshold of acetaldehyde in natural water ranges from 20 to 40 ppb (Nijssen *et al*., 1996). Fazzalari (1973) reported the flavor threshold for acetaldehyde in mineral water as 15 ppb, while Poretta and Minuti (1995) reported the flavor threshold for natural and carbonated water as 15 and 36 ppb respectively.

The FDA (1988) generally recognized acetaldehyde as safe. Even though inhalation studies using experimental animals have shown that pure acetaldehyde is capable of inducing nasal carcinomas (Feron *et al*., 1982), concentrations up to 125 mg/kg body weight/day show no adverse effects of hyperkeratosis (Til *et al*., 1988).

Methods of analysis

The extreme volatility of acetaldehyde makes it difficult to analyze. Gas chromatography (GC) has been most commonly used for the analysis of volatile flavor compounds. Generally, volatile flavor components of milk and cheese are present in trace amounts and require isolation and concentration for GC analysis (Chin *et al*., 1996). Different analytical approaches have been applied for isolation and studying of volatile components over the years (Chin *et al*., 1996; Yang and Peppard, 1994). These include simultaneous distillation-extraction, molecular distillation, solvent extraction, dynamic headspace sampling, static headspace methods, membrane dialysis, conventional solid-phase extraction and purge-and-trap sampling. Among conventional GC sample preparation methods, the static headspace appears to be the least costly and simplest. It also provides a volatile profile similar to the aroma perceived by the nose. The other methods generally require solvents or special apparatus, and are relatively time-consuming (Chin *et al*., 1996).

Nijssen *et al*. (1996) suggested headspace gas chromatography for analyzing acetaldehyde in mineral water where the sensitivity can be increased by injecting larger volumes of headspace. A detection limit of acetaldehyde in water of 1 ppb can be obtained. Improta *et al*. (1984)

determined acetaldehyde as cyanohydrin derivative by gas chromatography with a nitrogen-phosphorous detector. The reported detection limit was 100 ppb. Liquid chromatography was used by Edlund (1987) for determining acetaldehyde as dinitrophenylhydrazone with a detection limit of ~1ppb. Takami *et al*. (1985) combined an extraction and a derivatization method, where a cartridge was packed with a cation exchange resin charged with 2,4-dinitrophenylhydrazine. Water (500 ml) was passed through and the derivatives were selectively eluted with acetonitrile. When the eluate was analyzed by high performance liquid chromatography, 0.3 ppb acetaldehyde could be determined.

A relatively new variation of adsorption technique called solid-phase micro-extraction (SPME) has been developed by Arthur and Pawliszyn (1990) and Arthur *et al*. (1992) for the extraction of volatile and semi-volatile compounds from waste water samples. The SPME method is based on adsorption of analytes on a suitable fiber coated with a sorbent. This fiber can be immersed in the liquid sample (SPME liquid sampling) or exposed to the headspace above the sample (SPME headspace sampling). When the SPME fiber is immersed in the liquid sample, a partitioning of the compounds in the sample between the aqueous phase and the fiber surface occurs. The adsorbed compounds can then be thermally desorbed in a GC injection port (Arthur and Pawliszyn, 1990). Compound identification can then be based on comparison of GC retention indices and mass spectra with those of authentic compounds (Yang and Peppard, 1994).

Although SPME was originally developed for the analysis of pollutants in water samples, it has been more recently applied for the analysis of volatile flavor compounds in foods and beverages. Hawthorne *et al*. (1992) successfully applied SPME with an uncoated fused silica fiber for determination of caffeine in beverages. Marsili (1999) used SPME-GC/MS for the analysis of light-induced lipid oxidation products in milk and Yang and Peppard (1994) compared liquid and headspace SPME sampling.

Marsili (1999) compared SPME and dynamic headspace (DH) method for GC/MS analysis of light-induced lipid oxidation products in milk. He found that SPME is not only less expensive, but has better precision and accuracy, lower coefficients of variation between samples, and the linearity of calibration curves is consistently better.

Yang and Peppard (1994) examined liquid and headspace SPME sampling in a test solution comprising 25 common flavor components and applied this technique to the analysis of authentic food, beverage and flavor samples. A detection limit of the order of 0.1-10 ppb was estimated for most of the components. When conditioned before use, the SPME fiber generated only low background noise in gas chromatograms. The amount of an analyte adsorbed on the SPME fiber, and the resulting sensitivity, are determined both by adsorption kinetics and by the distribution coefficient of the compound between the fiber surface and the sample. Unlike conventional solid-phase extraction and purge-and-trap sampling techniques, in which a practically quantitative recovery is often achieved, SPME is more sensitive to experimental conditions. Any change of experimental parameters, which affect the distribution coefficient and adsorption rate, will also influence the amount adsorbed on the SPME fiber and the corresponding reproducibility (Yang and Peppard, 1994).

Although SPME adsorption is generally not quantitative, the concentration change of the sample after SPME adsorption cannot be ignored, especially if a small sample volume is used. Yang and Peppard (1994) found that when larger gas phase volumes (5 – 6 ml) were sampled from small sample solutions (0.5 ml), a decrease in relative FID response occurred.

SPME provides many advantages over conventional sample preparation techniques. It is very simple, rapid, uses no solvent for extraction (sample components cannot be hidden under a solvent GC peak), are relatively low cost, and does not result in dilution of the volatiles (Yang and Peppard, 1994; Marsili, 1999).

Implications of processing and storage on vitamin and volatile compound concentrations in milk

Among storage conditions, time, temperature and exposure to light play important roles in off-flavor development and vitamin degradation in milk. Rate of chemical reactions in milk products increases at higher storage temperature and with time, resulting in increased volatile compounds, which lead to flavor changes (Bassette and Jeon, 1984; Rerkrai *et al*., 1987; Cadwallader and Howard, 1998; Cladman *et al*., 1998; Jung *et al*., 1998; and Marsili, 1999).

Jeng *et al*. (1988) confirmed the mechanisms of light-oxidation flavor in milk. Photoreduction of riboflavin in milk results in the Strecker degradation of methionine to form potent odorant methional. It also leads to the photogeneration of superoxide anion, which can subsequently undergo dismutation to form singlet oxygen that can initiate oxidation of polyunsaturated fatty acids, leading to formation of numerous volatile carbonyl compounds.

Rerkrai *et al.* (1987) studied the effect of various direct ultra-high temperature heat treatments on the flavor of commercially prepared milks. Cooked flavor declined sharply in samples stored at room temperature while it declined more slowly at refrigerated temperatures. Stale flavor intensity increased in all samples throughout storage, but at room temperature it appeared sooner and was more pronounced than at refrigerated temperature. Rerkrai *et al*. (1987) also identified four aldehydes, acetaldehyde, propanal, n-butanal, and n-hexanal in UHT-milk and monitored their concentrations for changes during storage. Acetaldehyde and n-hexanal concentrations increased noticeably from week 7 of room temperature storage to the end of the study period, whereas at refrigerated temperature storage levels increased only slightly. Higher heat treatments produced greater concentrations of acetaldehyde.

Rerkrai *et al.* (1987) concluded that aldehydes were more important in contributing to off-flavor in UHT milk than ketones. The increases in acetaldehyde, propanal, and n-hexanal were time-temperature dependent during storage and paralleled stale flavor development, which might indicate that lipid oxidation was operative but not to the point at which a typical oxidized flavor was detected.

Bassette and Jeon (1984) concluded in a similar study to Rerkrai *et al*. (1987) that acetaldehyde and n-pentanal increased more rapidly in milk sterilized at 154^{o}C / 3.4 s than 146^{o}C / 3.4 s, whereas n-hexanal concentrations were lower in milk sterilized at 154^{o}C / 3.4 s. Further, Bassette and Jeon (1984) agreed, in general, with the conclusions of Rerkrai *et al*. (1987) concerning the time-temperature dependent reactions during storage and paralleled stale flavor development.

Grieg and Manning (1983) studied the changes in acceptability of pasteurized milk stored under refrigeration and how the volatile compounds in the milk related to its quality and age. Acetaldehyde was the only major component in the milk headspace to change concentration in a manner consistent with age. It was shown to have a strong positive correlation with consumer acceptability ($r = 0.95$). As acetaldehyde concentrations increased in the milk, consumer acceptability decreased.

Bassette *et al.* (1963) showed the addition of acetaldehyde to fresh milk in 0-30 ppb quantities did not decrease consumer acceptability, but the distribution of acetaldehyde between the different phases in milk might have been different from that produced naturally. They suggested acetaldehyde is produced via a chemical process presumed to be oxidation of a non-fat component of milk. Maarse (1991) indicated acetaldehyde that occurs naturally in milk is produced through the cow's metabolism, even when the feed is an odorless synthetic diet, and acetaldehyde development during storage occurs due to the oxidative breakdown of unsaturated fatty acids, particularly those present in the phospholipids. Volatile secondary oxidation products (particularly saturated and unsaturated aldehydes and ketones) are the main cause of oxidation off-flavors (Maarse, 1991).

Bills *et al.* (1972) detected a decrease in acetaldehyde concentration in yogurt stored under refrigeration for 2 weeks. They concluded that this decrease could be due to the ability of numerous lactic organisms to reduce acetaldehyde to ethanol.

Extended exposure to fluorescent or ultra violet light may alter the flavor and nutritive value of dairy products, depending on the intensity and wavelength of the light source, the translucency of the packaging material, and the duration of exposure. It causes destruction of key ingredients such as riboflavin, ascorbic acid and the essential amino acid methionine, and creates flavor defects called "light-induced oxidation" (Chapman *et al.*, 1998; Cladman *et al.*, 1998). Two reaction mechanisms are involved in the development of light-induced off-flavors. The first is a light-activated and riboflavin-catalyzed breakdown of serum proteins (peptides), resulting in the formation of volatile thiols, sulfides and disulfides (methanethiol, dimethyl sulfide, dimethyl disulfide), and 3-methylthiopropanal (methional) by degradation of methionine. As a result,

flavor defects occur that are described as cabbage or burnt protein and predominate for approximately two or three days (Maarse, 1991; Marsilli, 1999). The second pathway contributing to light-induced off-flavors is lipid oxidation. These flavors develop as a result of oxidative breakdown of unsaturated fatty acids, particularly those present in the phospholipids. Volatile secondary oxidation products (particularly saturated and unsaturated aldehydes and ketones) are the main cause of oxidation off-flavors. The resulting off-flavors are best described as metallic or tallowy (Maarse, 1991).

Bassette *et al.* (1983) studied the effect of pasteurization temperature on susceptibility of milk to light-induced flavor. Milk was pasteurized at different temperatures, homogenized, packaged in clear glass bottles or foil-covered glass bottles and then exposed to fluorescent light. Milk pasteurized at 73^{o}C and held in foil-covered bottles through 10 days at 2^{o}C had the most acceptable flavor. At pasteurization temperatures of 80^{o} and 90^{o}C, the adverse effect of irradiation was either reduced or eliminated and the incidence of oxidized flavor lessened. Concentration of acetaldehyde, propanal, n-pentanal and n-hexanal increased much more in the light-treated samples than those kept in the dark. However, high-heat treatment (90^{o}C) lessened those increases in propanal and n-hexanal but enhanced increases in acetaldehyde and n-pentanal (Bassette *et al*., 1983).

Acetaldehyde migration from packaging to product

Plastic polymers, such as HDPE and PETE have been used in contact with food products for many years. Milk is currently packaged in HDPE containers and PETE is extensively used for carbonated soft drinks, mineral waters, beers, wines, spirits and edible oils (Nijssen *et al*., 1996).

It has been found that residual monomers, such as ethylene glycol or terephthalic acid and isophthalic acid, oligomers and degradation products, such as acetaldehyde, and catalyst residues may migrate from PETE to the product (Nijssen *et al*., 1995). Many factors play a part in the migration process of compounds from the package to the foodstuff. The most obvious is the nature of the foodstuff itself, that is its fat or alcohol content and acidity, and the time and temperature of exposure. Other factors depend on the geometry of the polymer e.g. orientation

and molecular weight or on the geometry of the package e.g. thickness and surface to volume ratio (Ashby, 1988; Suttles and Marshall, 1993).

The migration of compounds may influence the sensory quality and acceptability of the foodstuff. In particular, the odor of mineral water stored in PETE bottles compared to that of soft drinks, can be detected at very low levels, due to the absence of masking flavor compounds (Nijssen *et al*., 1996).

PETE is a polyester resin with a partially aromatic structure, obtained from terephthalic acid and ethylene glycol. Its considerable mechanical resistance, as well as its characteristics of lightness, transparency and gas tightness, together with comparatively low costs, make this polymer particularly suitable for the commercial production of bottles for mineral water and drinks (Poretta and Minuti, 1995). Bottles made from PETE provide excellent barrier properties and higher heat stability (Nijssen *et al*., 1996). One disadvantage linked to the use of PETE in the production of bottles for mineral waters and soft drinks is the release of acetaldehyde from the container into the product (Poretta and Minuti, 1995).

Several studies have been done on the migration of acetaldehyde from PETE. Ashby (1988) used water as a food simulant in an experiment to determine the migration of acetaldehyde from PETE packaging to the product and found that the acetaldehyde concentration was <50 ppb after 8 days at 55^{o}C.

Nijssen *et al*. (1996) did a survey on acetaldehyde development in water packaged in PETE. The increases of acetaldehyde in uncarbonated and carbonated water stored in 1 L glass bottles, one-way 1.5 L PETE bottles, and unused refillable 1.5 L heat-set PETE bottles were monitored. They found that in uncarbonated water no acetaldehyde could be found, whereas the concentration of acetaldehyde in carbonated mineral water increased steadily upon storage, more so at 20^{o}C than at 30^{o}C, up to a level of 100 ppb. The fact that no acetaldehyde is found in uncarbonated water when stored in PETE bottles can be partly explained by Hagemeyer (1978). Acetaldehyde can be readily oxidized by oxygen to acetic acid, acetic anhydride and peracetic acid, or reduced to ethanol by catalysts such as nickel and copper oxide and easily transformed

by acids into the trimer paraldehyde. The availability of oxygen for oxidative reactions is also of great importance in light-induced off-flavor development in milk. Milk normally contains in the order of 0.07 volume % oxygen (Johnson, 1978). The permeability of the packaging material thus is important also since high oxygen transmission rates could lead to constant availability of oxygen for oxidation.

Jung *et al*. (1998) used sensory evaluation and dynamic headspace analysis and gas chromatography to conclude that dimethyl disulfide is mainly responsible for light-induced off-flavor in skim milk. They support the mechanism that dimethyl disulfide is formed by singlet oxygen oxidation of methionine, as found by Maarse (1991). Jung *et al*. (1998) also showed that the singlet oxygen quencher, ascorbic acid (200, 500, and 1000 mg/L levels) lower the formation of dimethyl disulfide and off-flavor development in milk.

Cladman *et al*. (1998) compared the effectiveness of clear PETE, green PETE, clear PETE with UV block, HDPE jugs, and low-density polyethylene pouches for chemical changes in milk over a period of 18 days. Green PETE showed best protection of milk against lipid oxidation, with clear PETE showing the worst results. Clear PETE containers with UV block showed little protection against lipid oxidation.

Cadwallader and Howard (1998) exposed milks of varying fat levels (skim, 2 % and whole) to fluorescent light for 48 hours and found that light-induced flavor in milk is impacted by the fat level of the milk. All levels showed "mushroom", "plastic", and "butterscotch" flavors, which they have said to be due to 1-octen-3-one and 1-hexen-3-one. Whole and 2 % milk had a distinctive "butterscotch" note, while this flavor was weak in skim milk.

Kim and Morr (1996) recovered hexanal, pentanal, dimethyl disulfide, 2-butanone, and 2-propanol from milk exposed to fluorescent light for 48 hours at 0 – 5^{o}C. Formation of most of these compounds was favored by providing headspace during light exposure. This is once again an indication that oxygen transmission rates of containers will play a big role in light-induced off-flavor development in milk exposed to light.

Leong *et al*. (1991) studied the presence of "plastic packaging flavor" in milk with various fat content, packaged in polyethylene-coated paperboard cartons. They suggested that this off-flavor is due to oxidative changes on the PE surface and that the flavor compounds responsible therefor is largely soluble in water and quite volatile. They concluded that it was easier to detect packaging flavor in skim milk than in whole milk and suggested that milk fat masks or dilutes the flavor defect.

For some time it has been known that light causes destruction of vitamins. Of the nutrients found in milk, laboratory studies have been concerned mainly with the stability of vitamin A, riboflavin and ascorbic acid. Sattar *et al.* (1977) found that vitamin A was rapidly destroyed by wavelengths below 415 nm and unaffected by wavelengths above 455 nm, while ascorbic acid and riboflavin is affected by wavelengths in the 400 – 500 nm range (Hansen *et al*., 1975).

Fanelli *et al*. (1985) investigated the effectiveness of visible and UV light screens in the protection of vitamins in milk from photodegradation. They found good protection of vitamin A and riboflavin by 0.3 wt % FD&C yellow #5, while the UV absorbers, Cyasorb 531 and Tinuvin 326, only offered protection of vitamin A.

Acetaldehyde as inhibitor against pathogens

It has been proven that acetaldehyde, in high concentrations, has inhibitory effects on some spoilage and pathogenic bacteria in milk. Bhalla *et al*. (1985a) concluded that acetaldehyde was 51.8% inhibitory against *Staphylococcus aureus* at concentration of 10, 000 mg/L. At concentrations of 10 mg/L, inhibitions were marginal and at 100 mg/L inhibitions were perceptible and progressed with the period of incubation up to 12 h. Thereafter, there was a decline in the degree of inhibition.

Acetaldehyde showed more inhibitory action on the growth of *Escherichia coli* than on *Staphylococcus aureus* (Bhalla *et al*., 1985b). Bhalla *et al*. (1985a) also demonstrated the inhibitory effect of acetaldehyde on *Bacillus subtilus*. They found that of all the milk volatile

compounds, highest inhibition of growth of *Bacillus subtilus* was achieved with acetaldehyde (47.8 %) at concentrations of 1000 mg/L and higher.

Such work suggest that acetaldehyde may have an inhibitory effect on spoilage and pathogenic bacteria which could extend shelf-life of dairy products. However, even acetaldehyde concentration of 10 mg/L exceeds the human threshold values of 0.82 mg/L in milk (Bills *et al*., 1972), and will very strongly decrease acceptability of milk. In flavored milk product, these levels might be masked, and could therefore be used to achieve inhibitory effects.

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CHAPTER III

HUMAN FLAVOR THRESHOLD FOR ACETALDEHYDE IN MILK OF VARIOUS FAT CONTENT, CHOCOLATE MILK, AND SPRING WATER

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A. ABSTRACT

The implications of milkfat on detection threshold of acetaldehyde were determined on whole, lowfat, and nonfat milks, chocolate flavored milk, and spring water. Acetaldehyde threshold in milk of various fat content is of great importance to the dairy industry since acetaldehyde develops in milk during storage as a result of light-oxidation and it is also a degradation product of polyethylene terephthalate, a relatively new packaging choice for milk. Sensory threshold testing of all mediums was duplicated using a panel of 25 untrained subjects.

Although acetaldehyde is lipid soluble, there was no significant difference in acetaldehyde threshold in milk of various fat content. Thresholds ranged from 3,939 to 4,040 ppb. Fat content thus seems to play no role in the flavor threshold for acetaldehyde in milk. Chocolate flavored milk and spring water showed thresholds of 10,048 and 167 ppb respectively, which compares favorably with previous studies. This high threshold of acetaldehyde in chocolate flavored milk is most likely due to masking of acetaldehyde flavor by chocolate flavor.

Solid phase micro-extraction (SPME) was verified as an effective method for recovery of acetaldehyde in all mediums. Acetaldehyde could be detected as low as 200 and 20 ppb in milk

and water respectively when using a Carboxen-PDMS SPME fiber in static headspace at 45^{o}C for 15 min.

Key words: acetaldehyde, milk, threshold, fat content, solid phase micro-extraction

B. INTRODUCTION

Shelf-life and exposure to ultraviolet (UV) light are two factors that greatly influence off-flavor development in milk. UV light initiates a variety of chemical reactions that result in the increase in volatile compounds such as acetaldehyde, propanal, n-butanal, n-hexanal, dimethyl disulfide, and methional, often to levels above human threshold (Cadwallader and Howard, 1998). These chemicals can lead to unwanted flavor changes in milk, previously described as sunlight, oxidized, activated, burnt, scorched, cabbage and mushroom (Dimick, 1982; Rerkrai *et al*., 1987; Grieg and Manning, 1983). The concentration of these chemicals is also increased by higher storage temperatures and with time (Rerkrai *et al*., 1987; Grieg and Manning, 1983).

Acetaldehyde is also a degradation product of poly(ethylene terephthalate) (PETE) which is a polyester resin (Feron *et al*., 1994; Poretta and Minuti, 1995). PETE is becoming an increasingly popular packaging choice for milk and beverage products. The release of acetaldehyde from the container into the product is a concern in the use of PETE packaging in the food industry. Pure acetaldehyde possesses a pungent irritating odor but at dilute concentrations it gives a pleasant fruity, green apple-like flavor. It occurs in a large number of natural food products in concentrations up to 1000 mg/L (vinegar) (Van Straten *et al*., 1983). In the dairy industry acetaldehyde is most often identified with fermented products, especially yogurt (5-40 mg/L) (Bodyfelt *et al*., 1988). Freshly pasteurized milk does not contain acetaldehyde in large quantities (10 ppb) (Maarse, 1991). The flavor threshold for acetaldehyde in milk was determined by Bills *et al*. (1972) in lowfat milk (2% milkfat) as 800 ppb. Detection threshold can be defined as an energy level below which no sensation would be produced by a stimulus and above which a sensation would reach consciousness (Lawless and Heymann, 1998). At high concentration (inhalation), acetaldehyde has been reported to have mutageno-carcinogenic effects in laboratory animals, but chronic oral toxicity is not yet proven (Feron *et al*., 1982).

The objectives of this study were to determine: 1) the flavor threshold for acetaldehyde in whole (3.25% milkfat), lowfat (2% milkfat), nonfat (0.5% milkfat), chocolate milk (3.25% milkfat), and spring water; and 2) if solid phase micro-extraction is an effective method in acetaldehyde recovery.

C. MATERIALS AND METHODS

Milk of various fat content, chocolate flavored milk, and spring water were analyzed. An untrained panel of 25 people and three-sample alternate forced choice test series was used for sensory analysis of all mediums (Lawless and Heyman, 1998). Quantification of acetaldehyde was done on all mediums using solid phase micro-extraction method coupled with gas chromatography (SPME-GC).

(i) Milk processing

Fresh raw milk was obtained from the Virginia Polytechnic Institute and State University dairy farm. Milk was pre-warmed to 55^{o}C and separated into cream and skim milk using a pilot plant separator (Elecrem separator, model 1G, 292xG, Bonanza Industries, Inc., Calgary, Canada). Milk was standardized at various fat contents (0.5%, 2% and 3.25%) by adding cream to skim milk in appropriate proportions.

Milk was pasteurized at 63.3^{o}C for 30 min in a batch pasteurizer (Creamery Package, P50.8770, MFG Co., Chicago, IL) and cooled to 25^{o}C. Milk (2% and 3.25% milkfat) was homogenized at 17.2 MPa (13.8 MPa - 1st stage; 3.4 MPa – 2nd stage) on a laboratory scale homogenizer (APV Gaulin, model 15 MR, Everett, MA), and stored at 4^{o}C in appropriate containers until needed. Fat content of milk was determined each time by the Babcock method (Marshall, 1993).

(ii) Chocolate milk

Whole milk (3.25% milkfat), sucrose, cocoa, and stabilizer were mixed (Appendix A). Formulated milk was pasteurized at 81^{o}C for 30 min in a laboratory scale batch pasteurizer and cooled to 25^{o}C. Milk was homogenized at 17.2 MPa (13.8 MPa - 1st stage; 3.4 MPa – 2nd stage) on a laboratory scale homogenizer (APV Gaulin model 15 MR, Everett, MA), and stored at 4^{o}C in appropriate containers until needed. Fat content of milk was determined by the Babcock method (Marshall, 1993).

(iii) Water

Kroger brand spring water (Kroger Co., Cincinnati, OH) was used for all analyses.

(iv) Preparation of acetaldehyde-spiked samples

Acetaldehyde (≥99.5%) was obtained from Fisher Scientific (Cincinnati, OH). Milk and water samples were used for acetaldehyde threshold testing within one week of processing. All samples were spiked volumetrically with 10 levels of acetaldehyde, in geometric progression of concentration steps. Concentration steps for milk were: 0; 200; 400; 800; 1,200; 1,600; 3,200; 6,400; 9,600; and 12,800 ppb: for chocolate milk 0; 400; 800; 1,600; 3,200; 6,400; 9,600; 12,800; 19,200; and 25,600 ppb: and for water 0; 2.5; 5; 10; 20; 40; 80; 120; 160; 320 ppb (Appendix B: Dilution Chart). The milk was thoroughly mixed and stored in a sealed amber glass container at 4^{o}C until sensory testing.

(v) Quantification of acetaldehyde in milk and water

Concentration of acetaldehyde in spiked milk, chocolate milk, and water samples was determined. Eight milliliters of sample, 5 µL internal standard solution (10,000 µg/ml 4-methyl-2-pentanone; Fisher Scientific, Cincinnati, OH and a micro stirring bar were placed in a 20 ml amber glass container and capped with Black Viton septa (Supelco, Inc Bellefonte, PA). Samples were held at 4^{o}C until the next day when it was analyzed. The vial septum was prepierced in the center, if required, with a sharp thin probe just before analysis to facilitate insertion of the SPME needle. The SPME-fiber (Supelco, Inc Bellefonte, PA) was exposed, with the end of the fiber about 1 cm above the surface of the sample. The SPME unit was clamped in this position and magnetic stirring commenced. Acetaldehyde was adsorbed on a Carboxen-PDMS solid phase micro-extraction fiber (Supelco, Inc Bellefonte, PA) in static headspace at 45^{o}C for 15 min (Marsili, 1999).

After exposure was completed, the SPME unit was withdrawn from the septum and inserted into the injector port of the gas chromatograph. The injector temperature was 250^{o}C. The fiber was left in the injection port for 20 min before removing, minimizing the possibility of carryover.

Acetaldehyde was thermally desorbed in the injector port of a Hewlett Packard gas chromatograph (Model 5890A, Hewlett Packard, Avondale, PA) equipped with a HP 5895A ChemStation and a flame ionization detector. Separation was completed on a HP-5 capillary column (25 m x 0.32 mm, 1.05 μm) (Supelco, Inc. Bellefonte, PA) with gas (Helium) flow rates of 1.0 ml/min. Temperature program was 35^{o}C for 1 min, raised at 8^{o}C/min to 180^{o}C, and then after 1 min raised to 250^{o}C at 14^{o}C/min with final time of 3 min. All injections were made in the splitless mode. Acetaldehyde identification and quantification was based on retention time and peak area results for the standard solutions using the method of additions technique and an internal standard (Marsilli, 1999).

(vi) Sensory Testing

Sensory testing was done on all mediums to determine acetaldehyde threshold. A three-sample alternate forced choice test series was used with a panel of twenty-five people. The study was repeated twice to verify that results (thresholds) were within 20% of each other (Lawless and Heymann, 1998).

Each panelist was presented first with a warm-up sample at a supra-threshold level of acetaldehyde (12,800 ppb for milk, 320 ppb for water, and 25,600 ppb for chocolate milk) to familiarize the panelists with the expected taste of discrimination. The panelists were requested to complete the human subjects consent form (Appendix C) while resting from the warm-up sample.

Panelists were presented with 10 three-sample triangle sets (7^{o}C) in ascending concentration series. Triangle sets were presented on three trays, with three, three, and four sample sets respectively. Panelists were informed to choose the sample that tasted “different” within each three-sample set. If subjects responded negatively at the highest level or showed correct choices

at even the lowest levels, the individuals were retested to confirm the highest or lowest concentrations of detection (Lawless and Heymann, 1998).

Panelists were instructed to rinse with warm spring water between three-sample sets and were allowed to rest between trays to prevent fatigue. Panelists were not informed of the ascending concentration characteristics of the samples, although they might have acquired the knowledge by participating on multiple panels.

Each three-sample set included two samples of unspiked medium (milk, chocolate milk, or water) and one acetaldehyde-spiked sample at the given concentration. Each sample was coded with three-digit numbers to remove bias, and the position of the spiked sample within the three-sample set was randomized (Scorecard – Appendix D).

A panel of twenty-five people was randomly selected for testing of each milk product and water. Panelists were seated in individual sensory booths. Each panel was replicated twice.

(vi) Data evaluation

The threshold for acetaldehyde was interpreted in two ways, geometric mean threshold and logistic regression. The threshold of individual panelists were determined by taking the geometric mean of the last incorrect concentration and the first correct concentration (when all subsequent choices were correct) for each product (Lawless and Heymann, 1998). Geometric mean is the antilog of the mean of the log values for the last incorrect concentration and the first correct concentration step. Group threshold was calculated by taking the geometric mean of the individual panelists thresholds. Sensory analysis was duplicated to ensure that group thresholds was within 20 % of each as specified by Lawless and Heymann, (1998). Appendix G reports the panel responses for the determination of taste threshold. <0> indicates lack of detection of the spiked samples by the judges, while <1> indicates detection of an acetaldehyde-spiked sample.

Logistic regression is a technique for predicting the probability of "success" as a function of some predictor variable. In this context, the concentration of the acetaldehyde in the medium (x) is the predictor variable and a correct identification of a spiked sample is a success.

Let x= the concentration of the acetaldehyde in the medium and let p(x) = the probability that a panelist correctly identified a sample that contained acetaldehyde. The logistic regression model is:

$$p(x) = 1 / [1 + \exp(-\alpha - \beta x)]$$

where α and β are parameters that are estimated from the data. Data were analyzed using SAS (1988).

D. RESULTS AND DISCUSSION

Acetaldehyde detection can be measured in two different ways. By using logistic regression, the probability of "success" - the probability that acetaldehyde-spiked samples will be identified correctly - as a function of acetaldehyde concentration in the medium can be predicted. By using the geometric mean approach, the concentration of acetaldehyde, below which the subjects lack the sensitivity to detect the acetaldehyde in a sample, can be determined.

The use of solid phase micro-extraction as headspace extraction method for acetaldehyde in all mediums was verified as effective.

(i) Geometric Mean Approach

Individual taste thresholds are reported in Appendix G. Table 1 reports the group threshold of acetaldehyde in the different mediums. Thresholds for acetaldehyde in milk of different fat contents were very similar, with a difference of only 100 mg/L. Miyake and Shibamoto (1993) reported that fat is one of the major carriers of carbonyl compounds as well as acetaldehyde, therefore milk with higher fat content should contain more acetaldehyde. While a higher threshold value for acetaldehyde was found in whole milk as compared to nonfat milk in our study, the threshold values were not significantly different ($p<0.05$). Based on these results, fat content does not play a major role in the threshold of acetaldehyde in milk. Bills *et al*. (1972) found flavor threshold levels for acetaldehyde in lowfat milk (2% milkfat) at 5^{o}C of 800 ppb. This threshold is substantially lower than what was observed in our study, since they used trained panelists.

Table 1. Thresholds for acetaldehyde in unflavored milk (nonfat, low fat, whole), chocolate flavored milk and spring water as determined by the geometric mean approach

Medium[1]	Group threshold[2] (ppb)	Min/Max Individual Thresholds	% Variation between Replications
Nonfat milk	3,939	14 / 23,406	12.8
Low fat milk	4,020	283 / 23,406	3.8
Whole milk	4,040	14 / 23,406	2.5
Chocolate milk	10,048	566 / 46,757	2.1
Spring water	167	7 / 784	12.6

[1] nonfat milk (0.5 % milkfat), low fat milk (2 % milkfat), whole and chocolate milk (3.25 % milkfat)

[2] 25 panelists for each of two replications

Chocolate milk shows a group threshold for acetaldehyde of 10,048 ppb. This data compares well with studies done on strawberry flavored milk with a threshold of 11,700 ppb (Bills *et al*., 1972). Panelists were not able to identify acetaldehyde-spiked samples in chocolate milk at concentrations that were easily detected in unflavored milk. This could be ascribed to masking of acetaldehyde flavor by chocolate flavor (Bills *et al*., 1972).

Spring water showed a group flavor threshold of 167 ppb. This value compares well with previous studies done on flavor threshold of acetaldehyde in water. Over the last 30 years various research studies reported flavor thresholds for acetaldehyde in water ranging from 22 to 1300 ppb (Ahmed, 1978; Rothe and Wölm, 1967; and Berg, 1955). Nijssen *et al*. (1996) also reported an odor threshold used by the mineral water industry as ranging from 20 to 40 ppb.

Table 1 also shows great variability in individual thresholds of panelists. Lawless and Heymann (1998) confirm that individuals have very different abilities to detect flavor compounds, with some subjects “blind” to (unable to detect) certain flavors. It is important that such individuals are also included in group threshold tests, since they are part of the general public and will also consume the product.

Since individual thresholds vary substantially, it can influence group threshold a great deal, therefore valid threshold measurements require group threshold values with <20% variability between two replications (Table 1) (Lawless and Heymann, 1998).

(ii) Logistic Regression

Logistic regression and the geometric mean approach are measuring detection in two different ways. The geometric mean is based on the information of where the subjects' detection abilities break down, and logistic regression predicts where a certain percentage of the panelists will correctly identify the acetaldehyde-spiked milk.

Figure 1 shows the probability of correct identification of acetaldehyde-spiked sample in all mediums. Lawless and Heymann (1998) suggests an arbitrary level of 50% above chance guessing for determining threshold when an alternative approach, such as logistic regression is used. This level is calculated by making use of Abbotts's formula (Finney, 1971):

$$\text{Adjusted proportion correct} = (\text{observed proportion} - \text{chance}) / (1\text{-chance})$$

$$0.5 = (0.667 - 0.33) / (1\text{-}0.33)$$

The 50% above chance guessing for the triangle test thus requires 66.7% correct identification. For example, in low fat milk, the logistic predicts that at a concentration of 3,570 ppb of acetaldehyde, 66.7% of the panelists should be able to identify the milk that is spiked with acetaldehyde.

The probability of correct identification of acetaldehyde-spiked sample in all mediums at the threshold levels found when using the geometric mean approach is shown in Table 2. This means that at the threshold level for acetaldehyde in lowfat milk (4,020 ppb) 68.7% of the panelists used would be able to correctly identify an acetaldehyde-spiked sample. Thresholds calculated using logistic regression compared very well with thresholds from geometric mean calculation for lowfat, whole, and chocolate flavored milk, while nonfat milk and spring water showed thresholds with fair comparisons.

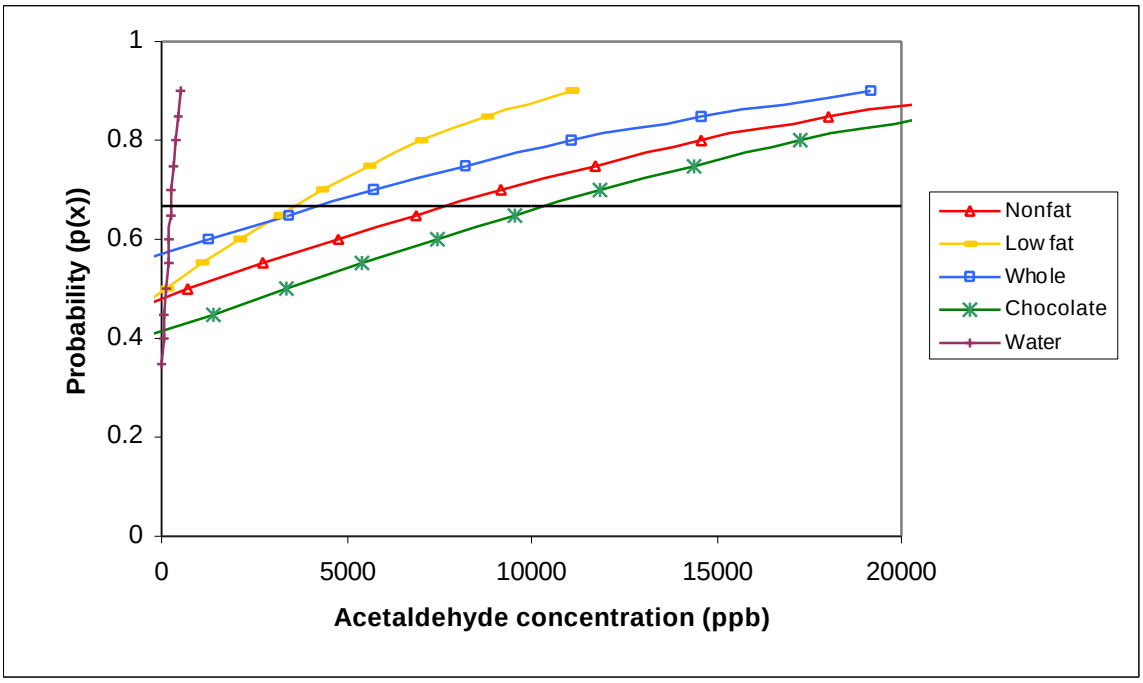

Figure 1. Probability of correct identification of acetaldehyde-spiked sample using logistic regression when p(x) = 1 / [1 + exp (-a–bx)]

Table 2. Predicted concentration of acetaldehyde at probability of 66.7 % using logistic regression as compared to group thresholds using geometric means

Medium	Acetaldehyde Rhreshold[1] At p(x) = 0.667 (ppb)	Geometric Mean Threshold[2]	Probability (%)[3] for Group Thresholds (ppb)
Nonfat milk	7,656	3,939	58.0
Low fat milk	3,570	4,020	68.7
Whole milk	4,174	4,040	66.4
Chocolate milk	10,334	10,048	66.1
Spring water	252	167	55.8

[1] Calculated at p(x) = 0.667 from logistic regression with p(x) = 1 / [1 + exp (-α–βx)]

[2] Calculated using geometric mean

[3] Probabilities calculated with p(x) = 1 / [1 + exp (-α–βx)] when using group thresholds obtained from geometric mean approach

(iii) Solid phase micro-extraction

Various analytical methods have been used over the years for the detection of acetaldehyde and other volatile flavor compounds in foods and beverages (Yang and Peppard, 1994; Marsili, 1999; Cadwallader and Howard, 1998). Dynamic headspace collection of volatiles coupled with gas chromatography is a sensitive technique, however, it is time-consuming especially in cleaning equipment and glassware and it involves relatively expensive equipment. Solid-phase micro-extraction coupled with gas chromatography (SPME-GC) is a solventless extraction technique that is simple, relatively cheap, and effective for isolating and detecting low levels of flavor compounds in foods and beverages (Yang and Peppard, 1994). Marsili (1999) used SPME to isolate various aldehydes in nonfat and low fat milk.

In our study solid phase micro-extraction method was an effective method for isolation and concentration of acetaldehyde from the headspace of the milk or water. Acetaldehyde was detected at concentrations as low as 200 ppb (Figure 2) and 20 ppb (Figure 3) in milk (3.25% milkfat) and water medium respectively when using a 75 μm Carboxen-PDMS fiber and a HP-5 capillary column (25 m x 0.32 mm, 1.05 μm).

Acetaldehyde shows a double peak in both mediums. This is most likely due to the volatility of acetaldehyde, which might prevent it from being optimally focused on the 1.05μm column used (personal communication, Robert Shirey, Supelco, Bellefonte, PA. November, 1999). In milk medium the retention times of the peaks are 1.175 and 1.236 min while they shift to 1.060 and 1.185 in water medium. This shift is unexpected, but was not researched further in this study.

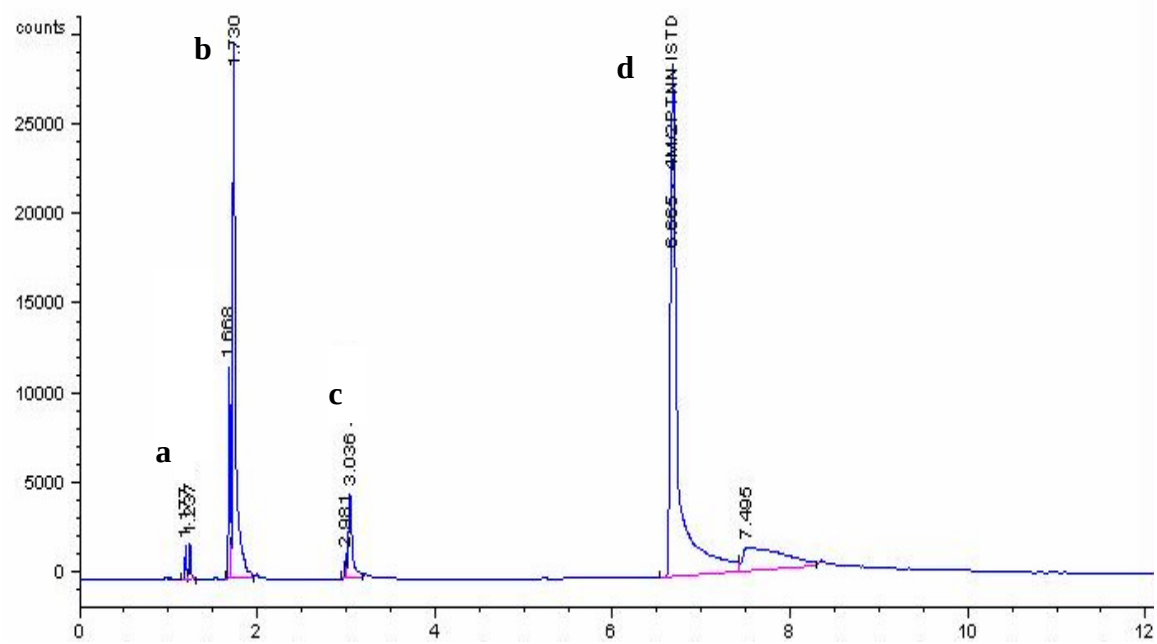

Figure 2. Acetaldehyde in milk medium (3.25% milkfat) at 200 ppb (a, acetaldehyde; b, acetone; c, n-butanone; and d, 4-methyl-2-pentanone (internal standard))

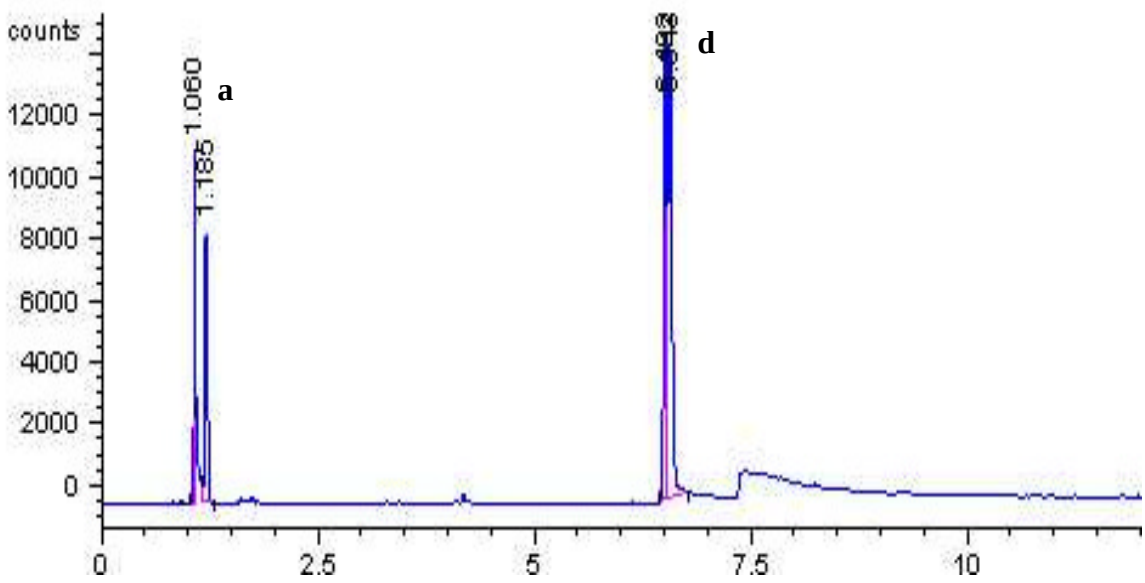

Figure 3. Acetaldehyde in water medium at 20 ppb (a, acetaldehyde; and d, 4-methyl-2-pentanone (internal standard))

Table 3 and 4 show recovery amounts of acetaldehyde in milk and water medium respectively. Recovered amounts in milk (3.25% milkfat) are higher in almost all cases. This could be due to the fact that acetaldehyde also is present naturally in milk at low concentrations (Maarse, 1991). Recovered and spiked amounts for water compared very well considering the low concentrations at which acetaldehyde was spiked and analyzed.

Calibration was based on peak area results for the standard solutions using the method of additions technique and an internal standard (4-methyl-2-pentanone) (Marsili, 1999). The calibration curve for acetaldehyde in milk (3.25% milkfat) and water showed a linear relationship between acetaldehyde concentration and area ratio of peaks with correlation coefficients of 0.999 (Figure 4) and 0.996 respectively. Nonfat, lowfat and chocolate flavored milk showed correlation coefficients of 0.997, 0.999 and 0.998 respectively.

Table 3. Acetaldehyde recovery from whole milk (3.25 % milkfat) using solid phase micro-extraction concentration technique

Spiked Concentration (ppb)	Recovered Concentration (ppb ± 100 ppb)[1]
200	280
400	511
800	904
1200	1169
1600	1492
3200	3331
6400	6351
9600	9792

[1] mean ± standard deviation

Table 4. Acetaldehyde recovery from spring water using solid phase micro-extraction concentration technique

Spiked Concentration (ppb)	Recovered Concentration (ppb ± 20 ppb)[1]
20	18
40	35
80	84
120	112
160	173
320	314

[1] mean ± standard deviation

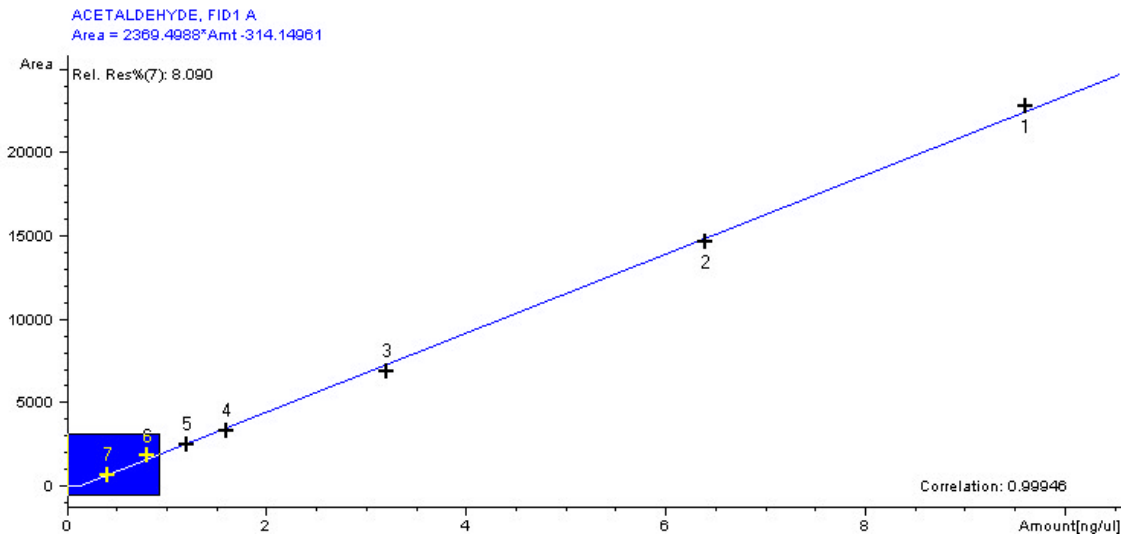

Figure 4. Calibration curve for acetaldehyde in whole milk (3.25% milkfat)

E. CONCLUSION

Acetaldehyde was found to increase in bottled water that was stored over time in certain plastic polymers, such as PETE (Nijssen *et al*., 1996). Since this packaging material is becoming an increasingly popular packaging choice for milk products and acetaldehyde is a degradation product of this polymer, it is important to know the flavor threshold for acetaldehyde in milk products.

Fat content does not play a role in the flavor threshold for acetaldehyde in milk. The detection threshold for acetaldehyde in chocolate milk (3.25% milkfat) was much higher (10,048 ppb) than for unflavored whole (3.25% milkfat) milk (4,040 ppb). This high threshold for acetaldehyde in chocolate flavored milk can be ascribed to the masking effect of the chocolate flavor. Water showed a flavor threshold of 167 ppb and 252 ppb when using the geometric mean approach and logistic regression respectively. These values compare well with results from previous researchers (Nijssen *et al*., 1996).

The geometric mean approach is easily influenced by incorrect responses by panelists as a result of fatigue or sensory adaptation. In contrast, logistic regression does not rely as strongly on individual responses.

Solid phase micro-extraction is a solventless extraction technique that is simple, relatively cheap, and effective for detecting low levels of flavor compounds in foods and beverages (Yang and Peppard, 1994). It proves to be an effective method for the recovery of acetaldehyde in milk, chocolate milk, and water. Levels as low as 200 and 20 ppb for milk and water mediums respectively have been confirmed.

F. ACKNOWLEDGEMENT

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CHAPTER IV

EFFECT OF SHELF-LIFE AND LIGHT EXPOSURE ON ACETALDEHYDE CONCENTRATION IN MILK PACKAGED IN HDPE AND PETE BOTTLES

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A. ABSTRACT

Whole milk (3.25% milkfat) was packaged in clear glass, high density polyethylene (HDPE), clear poly(ethylene terephthalate) (PETE), clear PETE with UV barrier, and amber PETE containers and exposed to fluorescent light at 1100-1300 lux (100–120 FC) for 18 days at 4°C. Two levels of light exposure (light-exposed or light-protected) were evaluated for each packaging treatment. Control (glass) and treated milks were evaluated by sensory and instrumental methods on day 0, 7, 14, and 18 of storage. Intensity of oxidation, acetaldehyde, and lacks freshness off-flavors was determined by eight experienced panelists.

No significant difference was found in acetaldehyde off-flavor between bottle treatments (exposed or protected). The concentration of acetaldehyde that developed over time in any of the containers also never exceeded flavor threshold levels for acetaldehyde in milk.

In light-exposed samples, oxidation off-flavor was significantly less when packaged in amber PETE versus the other containers. Milk packaged in HDPE containers also showed a significantly higher level of oxidation off-flavor on day 7 and 18 than milk packaged in PETE containers with UV block, but not from clear PETE or glass containers.

Lacks freshness off-flavor increased over time, as expected, in light-exposed and light-protected bottles and was more intense in the light-exposed containers over the first fourteen days.

Instrumental analysis (solid phase micro-extraction gas chromatography) showed increases in compounds associated with light oxidized flavor (acetaldehyde, pentanal, dimethyl disulfide (DMDS), and hexanal) in light-exposed samples over time.

Key words: acetaldehyde, oxidation, milk, polyethylene terephthalate, solid phase micro-extraction

B. INTRODUCTION

Light-induced off-flavors are one of the most common flavor defects in milk and have two distinct causes. The first, a burnt, sunlight flavor that develops and predominates for two or three days is said to be caused by degradation of sulfur-containing amino acids of the whey proteins (Marsilli, 1999). A metallic or cardboardy off-flavor then develops after two days and does not dissipate. This off-flavor is attributed to lipid oxidation (Barnard, 1972). Light exposure, especially to wavelengths below 500 nm, also causes the destruction of light-sensitive vitamins (riboflavin, vitamins A and C) (Fanelli *et al*., 1985).

The most common volatile compounds found in light-oxidized milk are dimethyl disulfide, pentanal, hexanal, 1-octen-3-one, acetaldehyde, and 1-hexen-3-one (Cadwallader and Howard, 1998; Cladman *et al*., 1998). These compounds are thus of great concern when analyzing light-oxidized milk. Light-induced flavor development is dependent on the availability of oxygen (Schröder, 1982). This can be controlled by reducing the amount of headspace in the container and by using oxygen impermeable containers to prevent further entry of oxygen. Poly(ethylene terephthalate) (PETE) thus has an advantage over high density polyethylene (HDPE) since the oxygen transmission rate (OTR) at 4^{o}C of a commercial PETE pint bottle is 19 μL/day compared to 390 - 460 μL/day for a commercial HDPE pint bottle (Personal Communication. Tom Clark, Eastman Chemical Co., Kingsport, TN. Nov 1999).

Acetaldehyde is a degradation product of PETE, thus making migration of this chemical from the package to the food product a possibility. Studies done on mineral water packaged in PETE containers showed migration of acetaldehyde from the package to the water (Nijssen *et al*., 1996). Since acetaldehyde is also a byproduct of light oxidation in milk, it is of utmost importance to make sure that acetaldehyde levels in the milk do not exceed human flavor threshold (Cladman *et al*., 1998; Cadwallader and Howard, 1998). The group flavor threshold for acetaldehyde in whole milk (3.25 % milkfat) is 4,040 ppb (Van Aardt, 2000). Levels above the threshold will influence the flavor of the milk.

PETE has many advantages over HDPE in which milk is packaged primarily. It has considerable mechanical resistance, it is light, transparent and relatively gas tight. Of great importance is that consumers will be able to see the product, which is not the case with pigmented HDPE.

The objectives of this study were to determine if there were differences in the development of certain off-flavors in milk as related to the packaging material (glass, HDPE, amber PETE, clear PETE with UV light block, and clear PETE). Sensory analysis as well as solid phase micro-extraction coupled with gas chromatography was used.

C. MATERIALS AND METHODS

Milk Processing and Packaging:

Milk (3.25% milkfat), processed as previously described (Chapter III), was packaged in different packaging materials (HDPE, amber PETE, standard clear PETE, and clear PETE with UV light block). Clear glass bottles were used as controls (Fisher Scientific. Cincinnati, OH). Eastman Chemical Co. (Kingsport, TN) provided the HDPE and PETE one pint bottles. Closures, made from low density polyethylene, were used. Aluminum foil, an inert material, was placed between the milk and closure on each bottle. All bottles were thoroughly rinsed and sanitized prior to use.

Milk storage and handling:

All samples were stored at 4^{o}C in a Tonka refrigeration unit (Hopkins, Minnesota), at the same level, under a row of fluorescent Econ-o-watt lights. Light intensity was 1100 - 1300 lux for all samples (Heer *et al*., 1995). Bottles were placed in randomized order in the refrigeration unit, to ensure even light distribution among various containers. After sampling, containers were refilled and placed back in the vacated positions in the refrigeration unit. This maintained the amount of light reflection among samples throughout the shelf-life duration. Two levels of light exposure (light-exposed and light-protected) were evaluated for each packaging treatment. "Light-protected" light exposure was obtained by covering the whole bottle body area with aluminum foil.

Two individual milk samples of each treatment were randomly chosen for evaluation on days 0, 7, 14, and 18. The entire study was replicated three times.

Product Evaluation

Microbiological quality:

In order to determine that milk used in the experiment was of similar microbiological quality and to verify that the type of packaging material did not affect the microbiological shelf-life of the product, all milk samples were microbiologically evaluated for standard plate count and modified psychrotrophic bacteria count on day 0 and 18 of each replication. Coliforms (Petrifilm: 32^{o}C for 48 h) were determined only on day 0 of each replication. When necessary, ten-fold dilutions were prepared in peptone water. Determinations were made according to standard methods (Marshall, 1993).

Sensory quality:

A panel of eight experienced people evaluated flavor characteristics (light oxidized, lacks freshness, and acetaldehyde) on days 0, 7, 14, and 18. Panelists were trained in four steps. First, the flavor of ideal milk was described according to Badings (1991). Secondly, each panelist was informed of the characteristics of three selected off-flavors and there was a discussion on the different defects and causes of these off-flavors. Acetaldehyde was described as having a pleasant fruity aroma or "green apple-like" flavor (Arctander, 1969; Bodyfelt *et al*., 1988). Oxidized flavor was described as burnt, scorched, cabbage, and mushroom (Dimick, 1982). Lacks freshness off-flavor was described as being not as pleasantly sweet and refreshing or as free of an aftertaste as is typically desired in milk. Thirdly, the principles of the 9-point verbal category test (1-not noticeable; 2-trace, not sure; 3-faint; 4-slight; 5-mild; 6-moderate; 7-definite; 8-strong; and 9-very strong) were described according to Lawless and Heymann (1998). Lastly, panelists familiarized themselves with the three off-flavors in 16 sessions over a period of four weeks. Training was done by repeatedly sampling off-flavors at levels: 2-trace, not sure; 4-slight; 6-moderate; and 8-strong (Appendix E). Selected panelists were retrained after the training period to ensure valid results.

Intensity of flavor was evaluated on a 9-point verbal category scale using a numerical scoring system of one for "not detectable" and nine for "very strong" for each attribute (Scorecard – Appendix F) (Lawless and Heymann, 1998). Samples (25 ml portions) in 2 oz amber glass bottles were coded with three-digit numbers and presented at 7^{o}C in random order to each panelist seated in individual sensory booths.

Sensory panel data were analyzed by ANOVA using the general linear model (GLM) procedure (SAS, 1988). Means were separated using Tukey's least squared means (LSM) method. Significance of differences was defined at $p<0.05$.

Chemical quality:

Volatile compounds associated with light oxidation and shelf-life such as acetaldehyde, dimethyl disulfide, hexanal and pentanal were measured quantitatively using solid phase micro-extraction coupled with gas chromatography (SPME-GC). These evaluations were done on day 0, 7, 14, and 18, in duplicate.

Eight-milliliter aliquots of all bottle treatments were pipetted into separate 20ml amber bottles and fitted with teflon-coated septa (Supelco, Bellefonte, PA) immediately after light exposure. Samples were held at 4^{o}C until the next day when analyzed by SPME-GC for acetaldehyde, pentanal, dimethyl disulfide, and hexanal.

Since the percent partition from the food matrix to the SPME fiber is reduced with increasing food lipid content, 2g sodium chloride per sample was added to the milk prior to extraction to increase the partition of volatiles into the headspace (Page and Lacroix, 1993; Zhang *et al*., 1994).

The vial septum was prepierced in the center, if required, with a sharp thin probe just before analysis to facilitate insertion of the SPME needle. The 75 μm Carboxen-PDMS coated SPME-fiber (Supelco, Bellefonte, PA) was exposed with the end of the fiber about 1 cm above the surface of the milk. The SPME unit was clamped in this position and magnetic stirring

commenced. After 30 min at 50°C the fiber was retracted, the SPME unit was withdrawn from the septum and inserted into the injector port of a Hewlett Packard gas chromatograph (Model 5890A, Hewlett Packard, Avondale, PA) equipped with a HP 5895A ChemStation. The volatile compounds were thermally desorbed by exposing the fiber, after which the oven temperature program started. Separation was completed on a HP-5 capillary column (25m x 0.32mm, 1.05μm), (Supelco, Inc. Bellefonte, PA) with gas (Helium) flow rates of 1.0 ml/min. The temperature program was 45°C for 0.5 min, raised at 9°C/min to 180°C for 0.5 min, and then raised to 240°C at 18°C/min with a final time of 5 min. The injector temperature was 280°C, and all injections were made using the splitless mode. The fiber was left in the injection port for 5 min before removing it.

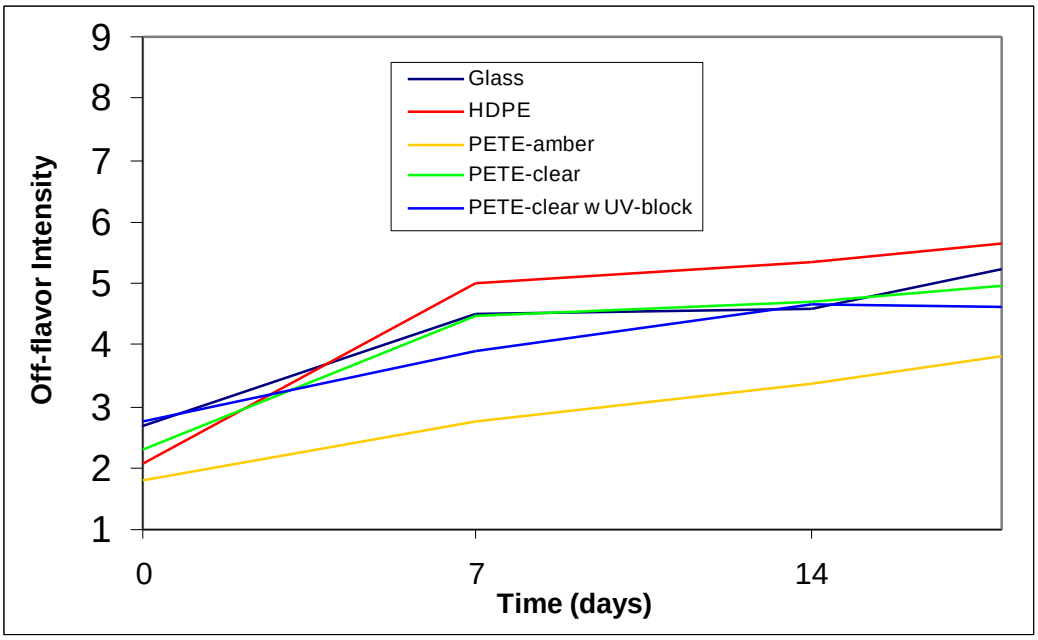
Volatile compounds in four independent milk bottles, for each bottle treatment, and each light treatment (light-exposed, light-protected) were evaluated on days 0, 7, 14, and 18. The reproducibility of the analyses was optimized by accurately controlling the temperature of the sample during adsorption and desorption. Since the concentration of the internal standard (4-methyl, 2-pentanone) deteriorated over time (old stock), quantification of compounds was not possible. The amounts of volatile compounds were determined by comparing area counts for each compound to that of the compound in the control (glass). Relative concentrations for acetaldehyde were determined by comparing area counts to a standard additions curve. Stock solutions consisted of concentration levels of 10; 100; 200; 400; 800; 1,200; 1,600; 3,200; 6,400; 9,600; and 12,800 ppb. Dimethyl disulfide, hexanal, pentanal, and acetaldehyde (99.5% pure) were obtained from Fisher Scientific (Cincinnati, OH).

D. RESULTS AND DISCUSSION

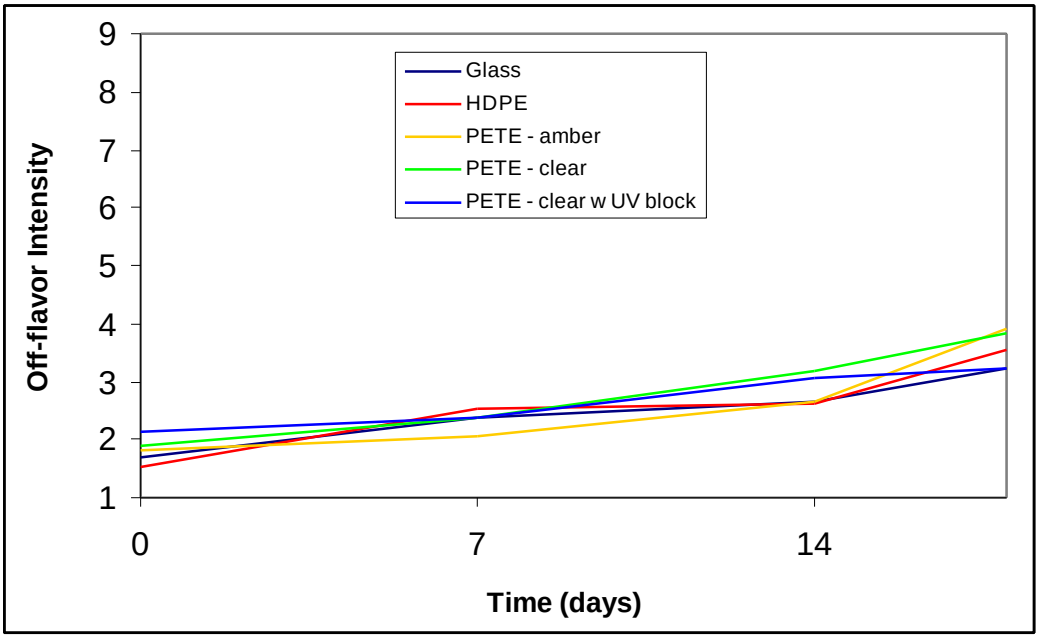
Oxidation off-flavors increased more rapidly in milk packaged in light-exposed containers versus light-protected containers (Figure 1). Of the light-exposed samples, milk packaged in amber PETE showed the least amount of oxidation off-flavor, since it blocks wavelengths below 450 nm and partially blocks wavelengths between 450 and 700 nm. This compares favorably with results found on protection of milk against light-oxidation when packaged in green PETE (Cladman *et al*., 1998). Volatile analysis demonstrated lower values for hexanal and dimethyl disulfide (DMDS) in milk packaged in amber PETE compared to all other packaging treatments (Table 1 and 2). The concentration of pentanal was also lower in milk packaged in PETE materials than in milk packaged in glass or HDPE (Table 3). On day 7 and 14 of shelf-life, hexanal, pentanal and DMDS could not be detected.

Milk packaged in clear PETE with UV light block showed less oxidation off-flavor on day 7 and 18 of storage than glass, clear PETE or HDPE containers (Figure 1). This is in agreement with light transmission rates of clear PETE, glass and HDPE (Figure 2) and a good indication that the UV block is partially protecting milk against wavelengths emitted by the fluorescent light source.

The relative area counts for the three volatiles evaluated (hexanal, dimethyl disulfide, pentanal; Table 1 – 3) on day 18 are very similar for milk packaged in clear PETE and clear PETE with UV block. Gas chromatograms demonstrate differences in unidentified volatiles for milk packaged in these two materials. The light block was not effective in minimizing the specific volatiles identified but appeared to have some effect against other volatiles that are associated with light-induced oxidation off-flavor (Figure 3 and 4).



(a)



(b)

Figure 1. Oxidation off-flavor in milk packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV block when (a) light-exposed and (b) light-protected (off flavor intensity: 1 – not noticeable; 9 – very strong)

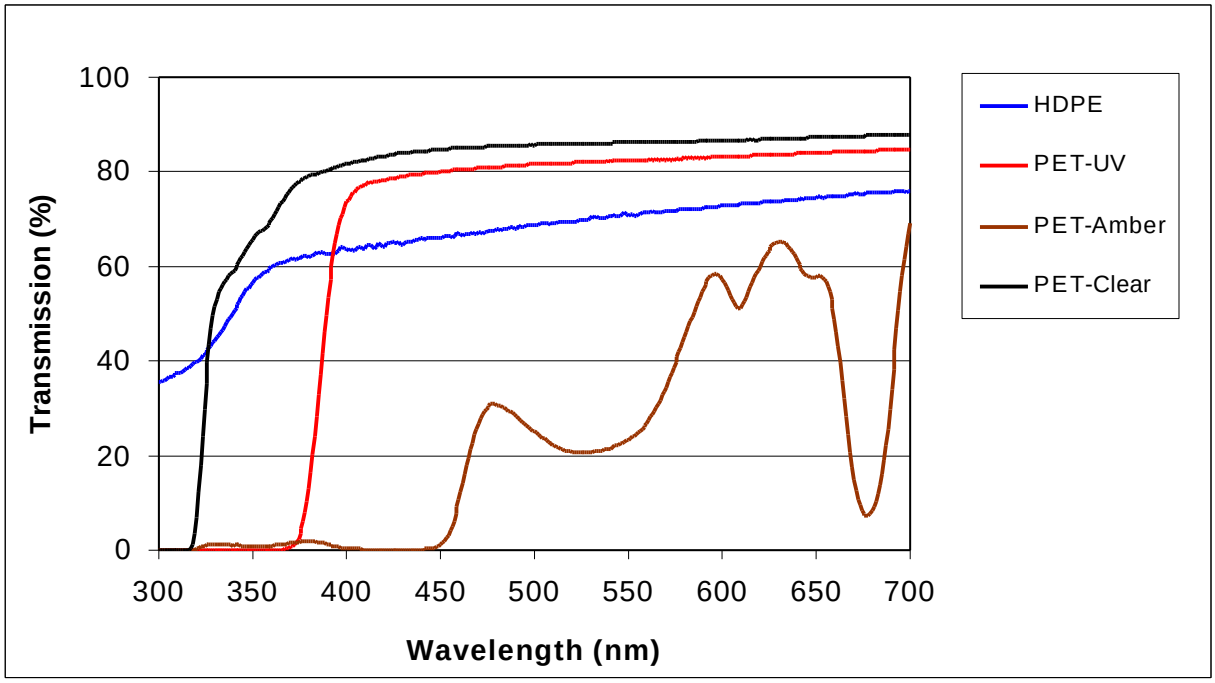


Figure 2. Light transmission spectra of clear PETE, amber PETE, clear PETE with UV light block, and HDPE

From Figure 1 (a), oxidation off-flavor seems much higher in milk packaged in HDPE containers, but the only significant differences were between HDPE and (i) clear PETE with UV light block on day 7 and 18, (ii) clear PETE and glass on day 14, and (iii) amber PETE. Since oxidation reactions are dependent on the availability of oxygen (Schröder, 1982), this difference in oxidation off-flavor could be due to the fact that the HDPE bottles contained more oxygen. This could be a function of the shape of the container versus that of the PETE containers or be due to a higher oxygen transmission rates than that of PETE containers (390-460 µL/day versus 19 µL/day at 4^{o}C respectively). HDPE also showed the highest amounts of volatile compounds when exposed to light (Table 1, 2, and 3). DMDS and hexanal developed to higher concentrations, as reflected by relating ratios, in milk stored in glass and HDPE containers, than in milk stored in the various PETE containers.

Table 1. Relative area ratios of hexanal in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block

	Day 0	Relative Area Ratio Day 18 (light-exposed)	Relative Area Ratio Day 18 (light-protected)
Glass	<0.05	1.0 ± 0.1	0.06 ± 0.002
HDPE	<0.05	1.14 ± 0.01	0.12 ± 0.002
Amber PETE	<0.05	0.39 ± 0.06	<0.05
Clear PETE	<0.05	0.74 ± 0.03	0.07 ± 0.014
Clear PETE with UV Block	<0.05	0.72 ± 0.04	0.05 ± 0.009

Table 2. Relative area ratios of dimethyl disulfide in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV block

	Day 0	Relative Area Ratio Day 18 (light-exposed)	Relative Area Ratio Day 18 (light-protected)
Glass	<0.05	1.0 ± 0.13	0.05 ± 0.004
HDPE	<0.05	1.52 ± 0.17	0.19 ± 0.041
Amber PETE	<0.05	<0.05	<0.05
Clear PETE	<0.05	0.53 ± 0.12	0.12 ± 0.037
Clear PETE with UV Block	<0.05	0.44 ± 0.09	<0.05

Table 3. Relative area ratios of pentanal in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block

	Day 0	Relative Area Ratio Day 18 (light-exposed)	Relative Area Ratio Day 18 (light-protected)
Glass	<0.05	1.0 ± 0.21	<0.05
HDPE	<0.05	1.07 ± 0.14	<0.05
Amber PETE	<0.05	<0.05	<0.05
Clear PETE	<0.05	<0.05	<0.05
Clear PETE with UV Block	<0.05	0.24 ± 0.08	<0.05

There was no significant difference detected in oxidation off-flavor in containers shielded from light. While relative concentrations of hexanal and DMDS remained low in all milk samples, milk packaged in HDPE showed higher amounts comparatively. Figures 3 and 4 show volatile compounds in fresh milk and light protected milk stored for 18 days in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV block.

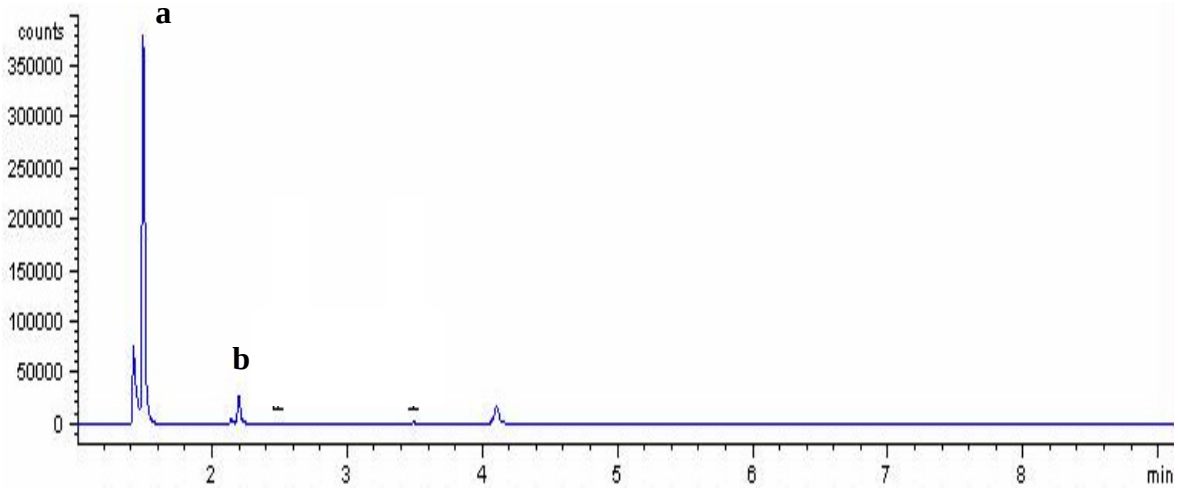
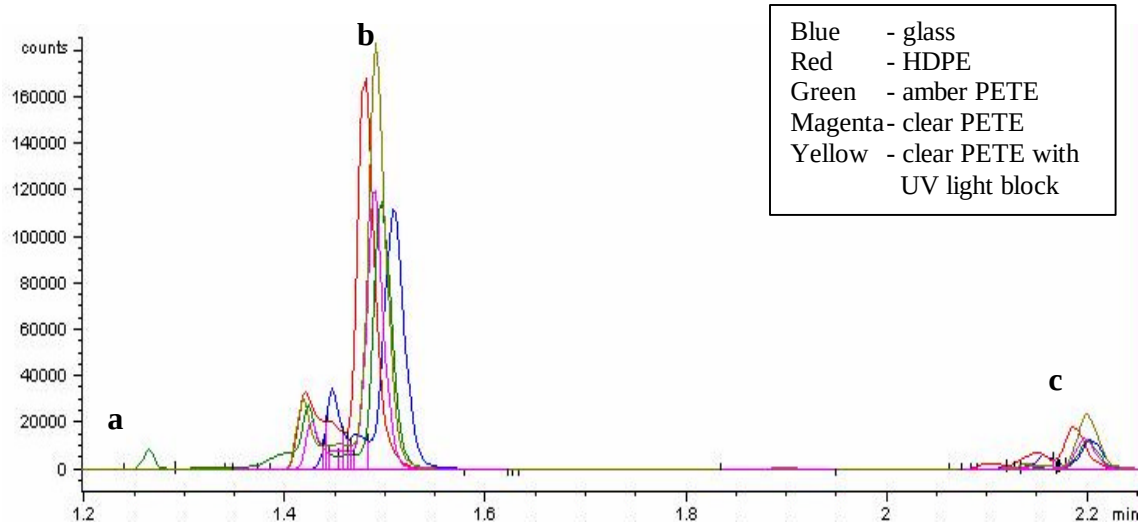


Figure 3. Volatile compounds in fresh milk (a, acetone; and b, n-butanone)



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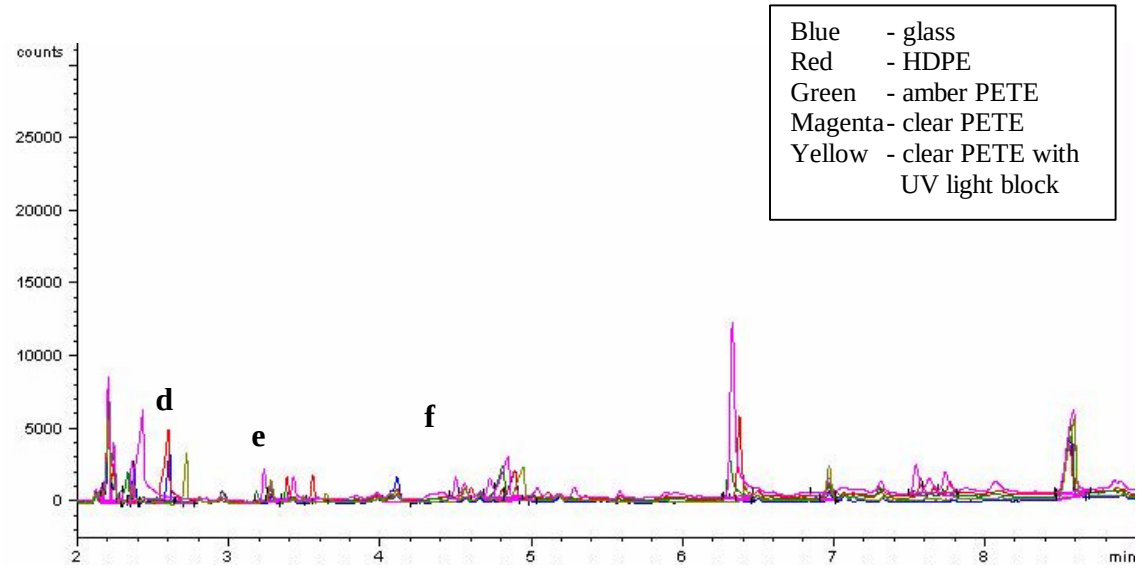
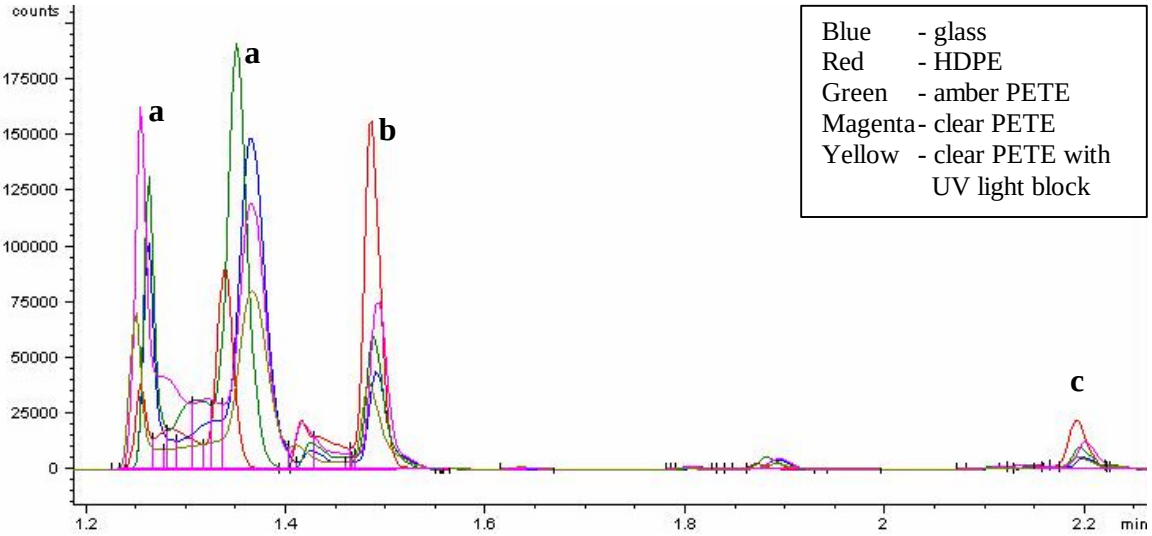


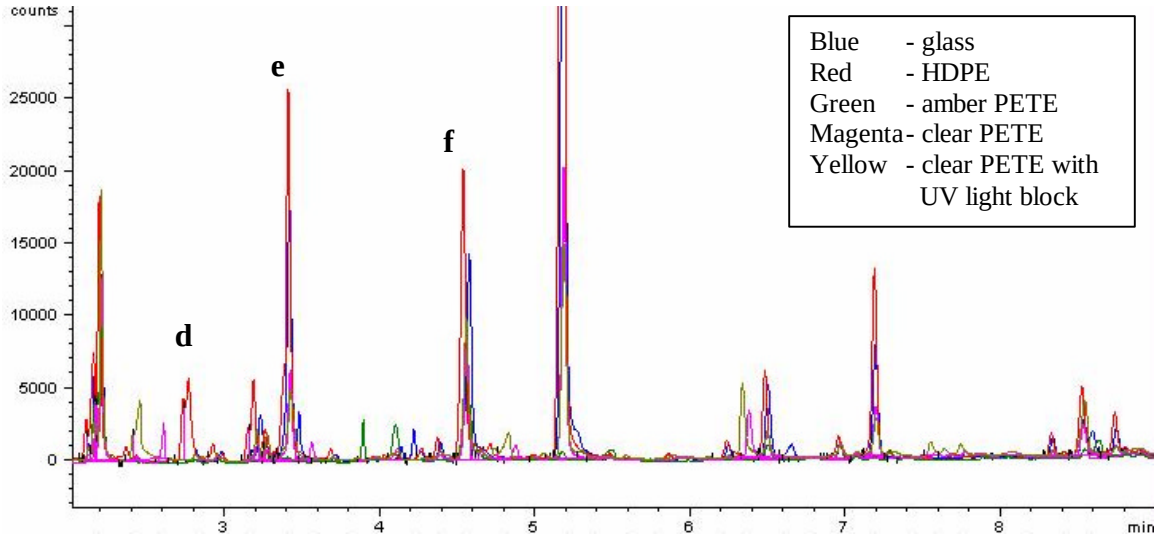
(b)

Figure 4. Volatile compounds in milk after 18 days of storage in glass, HDPE, clear PETE, clear PETE with UV block and amber PETE when protected against light (a) retention times 1.2 – 2.2 min and (b) retention times 2.2 – 9.0 min. (a, acetaldehyde; b, acetone; c, n-butanone; d, pentanal; e, dimethyl disulfide; and f, hexanal)

Figure 5 shows volatile compounds in light-exposed milk on day 18 of storage when packaged in glass, HDPE, clear PETE, clear PETE with UV light block, and amber PETE.



(a)



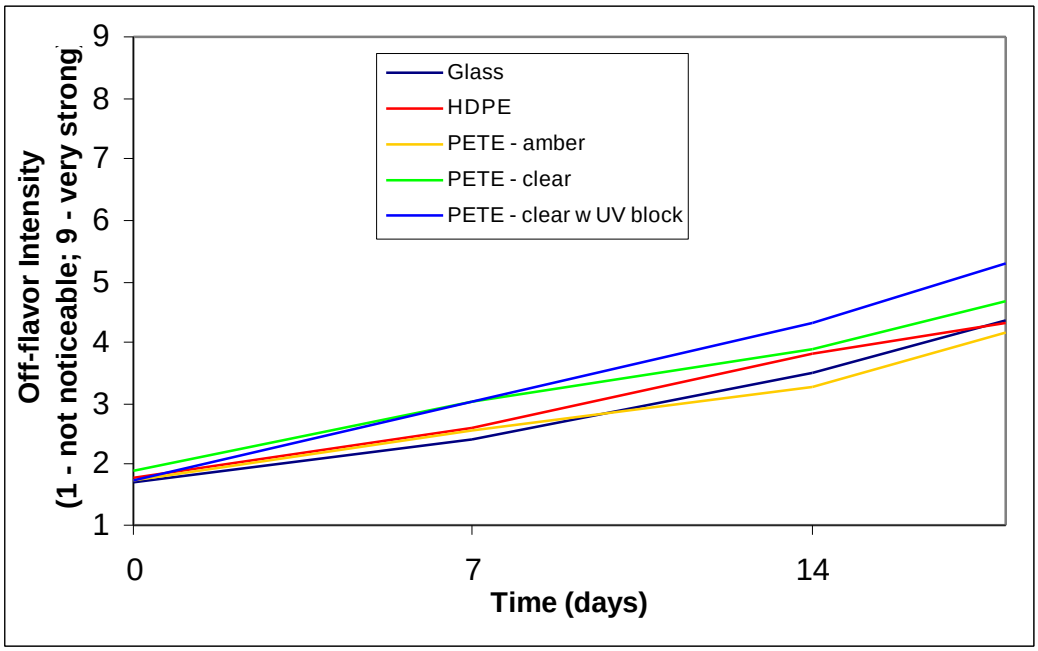
(b)

Figure 5. Volatile compounds in milk after 18 days of storage in glass, HDPE, clear PETE, clear PETE with UV block and amber PETE when exposed to light (a) retention times 1.2 – 2.2 min and (b) retention times 2.2 – 9.0 min. (a, acetaldehyde; b, acetone; c, n-butanone; d, pentanal; e, dimethyl disulfide; and f, hexanal)

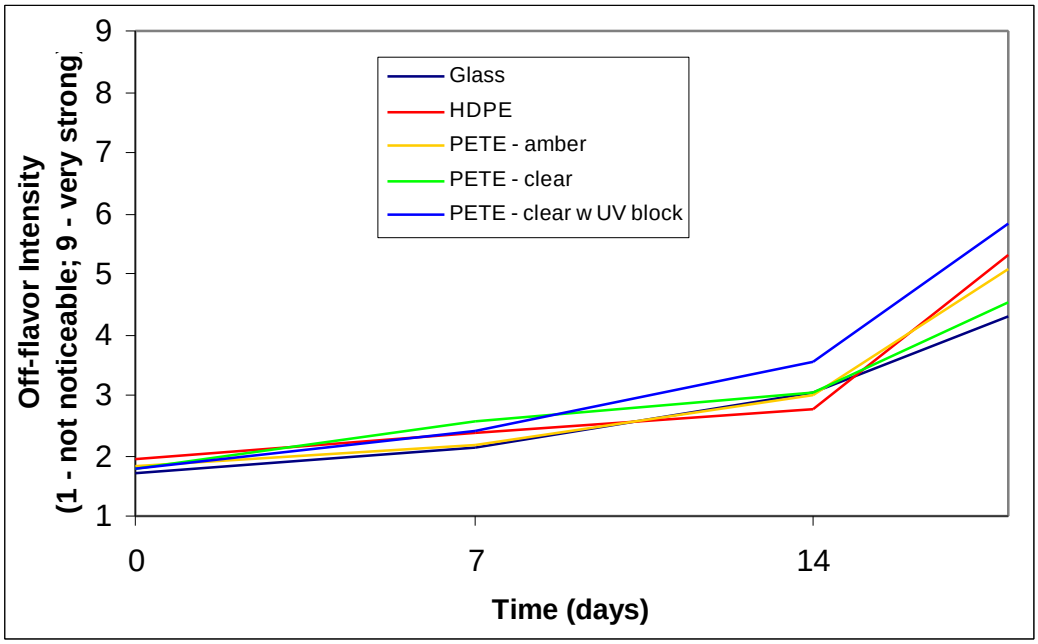
Lacks freshness off-flavor appeared to increase more steadily in light exposed packages than in light protected packages (Figure 6). The higher relative rate of increase in lacks freshness off-flavor may be attributed to at least two facts. Volatile compounds generated as a result of light oxidation may also be part of the lacks freshness profile. In addition, panelists may not clearly distinguish between light oxidation and lacks freshness, resulting in an increase in lacks freshness perception as light oxidation off-flavor also increases. Lacks freshness off-flavor did dramatically increase from day 14 to day 18 in light-protected milk. Panelists were more sensitive to differences when light oxidized off-flavor was not present.

The rate of microbiological growth in whole milk was monitored to ensure that milk flavor was not influenced differently by different bottle types, as well as to ensure that the milk quality never dropped below a level that was fit for human consumption. All samples were of comparable bacterial quality and within normal range on day 18 of shelf-life. Standard plate counts ranged between 5.3 – 7.8 log CFU/ml (Appendix G). At these overwhelming levels, strong off-flavors are expected, as shown in sensory analysis, and will to some extend mask any other off-flavors such as oxidation. All coliform counts were <1.0 log CFU/ml and aerobic bacteria counts and psychrotrophic bacteria counts were <20,000 CFU/ml on day 0 (Appendix G). These results fall within FDA specification (FDA, 1989).

Off-flavor as related to volatile analysis showed increases in certain peaks on the gas chromatogram at retention times 3.75 and 4.1 min (Figure 4). These peaks was more evident in light protected samples, thus being a good indication of lacks freshness byproducts. In this study they were not identified, but Cadwallader and Howard (1998) showed increases in 2,3-butanedione and other volatile compounds in milk when approaching the end of shelf-life.



(a)

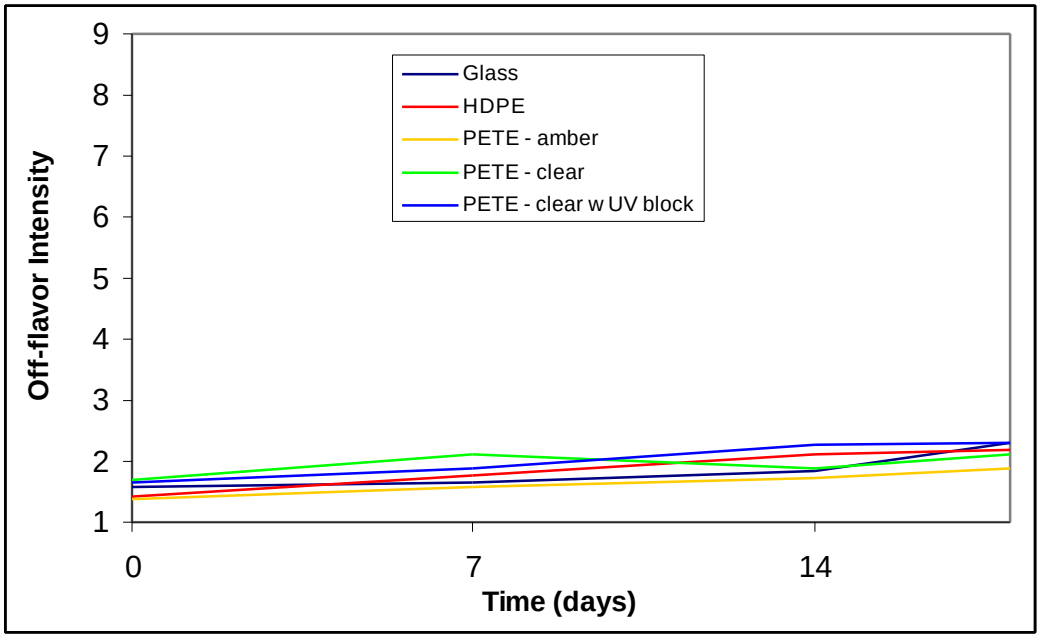


(b)

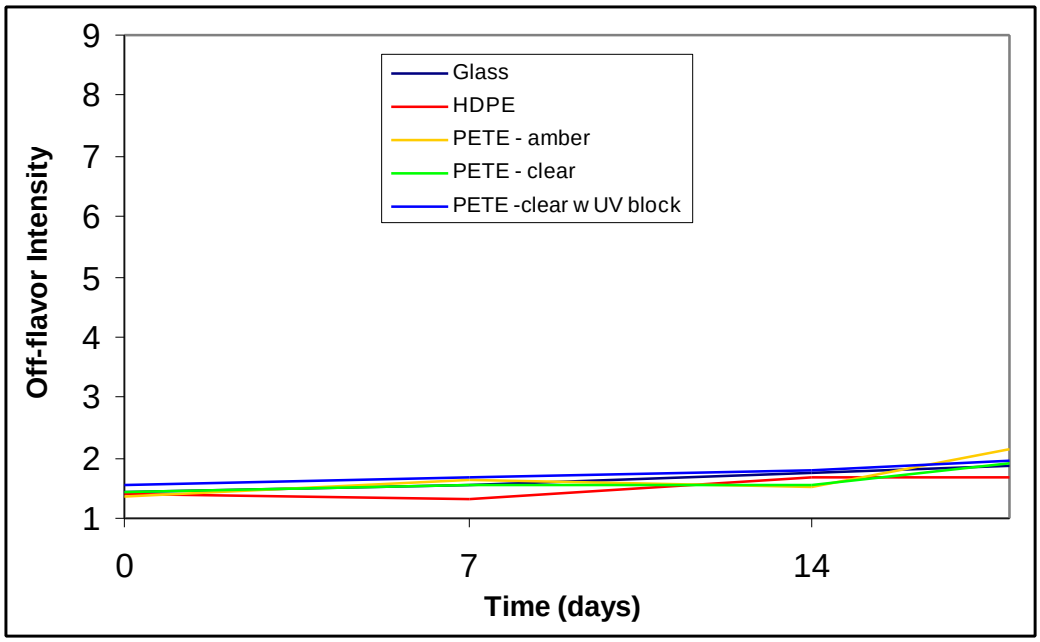
Figure 6. Lacks freshness off-flavor in milk packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV block when (a) light-exposed and (b) light-protected (off flavor intensity: 1 – not noticeable; 9 – very strong)

Light-exposed and light-protected milk showed the highest values for lacks freshness off-flavor when packaged in clear PETE with UV light block. This was only significantly higher than off-flavor development in other containers at day 14 and 18 of storage. These higher levels of lacks freshness off-flavor in clear PETE with UV block containers cannot be explained, but could be associated with the UV blocking agent. There was no significant difference in lacks freshness off-flavor among any of the other packaging materials.

Acetaldehyde did increase over time, and increased more rapidly when milk was exposed to light (Table 4). Acetaldehyde also seemed to increase more rapidly in milk packaged in PETE containers when exposed to light than in glass or HDPE, with the exception of clear PETE with UV block. The significantly lower level of acetaldehyde in HDPE containers could be due to various reasons. Acetaldehyde could leave the container due to high gas transmission rates of HDPE versus that of PETE or glass. It could adsorb onto the HDPE itself, since it is miscible in water and ethanol (Weast, 1976) or it could break down to other organic compounds, such as acetic acid, as a result of oxygen availability. Increases in acetaldehyde levels are only marginal in light-protected containers although the milk packaged in amber PETE had the highest acetaldehyde level on day 18. The low levels of acetaldehyde on day 18 in milk packaged in light-protected containers served as an indication also of the amount of acetaldehyde formed from microbial activity. None of the relative concentrations reported in Table 4 are above the human flavor threshold for acetaldehyde in milk (4,040 ppb) (Van Aardt, 2000). Since these concentrations were only relative, it is only an indication that human threshold is not exceeded. However, this fact correlates well with the fact that no significant amount of acetaldehyde was detected by panelists during sensory analysis (Figure 7). Panelists thus could not distinguish between acetaldehyde levels in milk packaged in the various materials.



(a)



(b)

Figure 7. Acetaldehyde off-flavor in milk packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV block when (a) light-exposed and (b) light-protected (off flavor intensity: 1 – not noticeable; 9 – very strong)

Table 4. Relative area ratios of acetaldehyde in fresh and light oxidized whole milk when packaged in glass, HDPE, amber PETE, clear PETE, and clear PETE with UV light block

	Day 0	Relative Area Ratio Day 18 (light-exposed) (Relative Conc. – ppb)	Relative Area Ratio Day 18 (light-protected)
Glass	<0.05	1.0 ± 0.26 (2220)	<0.05
HDPE	<0.05	0.57 ± 0.11 (1265)	<0.05
Amber PETE	<0.05	1.53 ± 0.07 (3397)	0.16 ± 0.02
Clear PETE	<0.05	1.32 ± 0.16 (2930)	0.05 ± 0.02
Clear PETE with UV Block	<0.05	0.79 ± 0.13 (1754)	<0.05

HDPE showed the lowest levels of acetaldehyde development in light-exposed and light-protected samples, while acetaldehyde was not detected by sensory analysis in any containers. This may be attributed to at least three facts. HDPE does not contain acetaldehyde as PETE does (Mathlouthi, 1994). Acetaldehyde is converted to acetic acid in the presence of oxygen. Since HDPE has the highest oxygen transmission rate (OTR) of the packaging materials studied, the container should contain the highest amounts of oxygen (Mathlouthi, 1994). In addition, the container will also allow the escape of acetaldehyde from the container.

E. CONCLUSION

Poly(ethylene terephthalate) (PETE) is becoming an increasingly popular packaging choice for milk products due to its transparency, good mechanical resistance and low gas transmission. Since acetaldehyde is a degradation product of PETE and also develops in milk during storage, sub-threshold levels of acetaldehyde are of great importance. High density polyethylene (HDPE), in which milk is currently primarily packaged, displays relatively high gas transmission rates. It provides low (opaque containers) to no (colored containers) visibility of the product in the container.

Panelists could not detect acetaldehyde in light-exposed or light-protected milk. Sub-threshold (threshold: 4,040 ppb in whole milk) levels of acetaldehyde were subsequently confirmed using solid phase micro-extraction coupled with gas chromatography. Therefore acetaldehyde migration from PETE packaging into milk over storage conditions of 4^{o}C and 18 days shelf-life should not affect consumer acceptability.

The best protection against light-oxidation, which increased in all light-exposed samples, was provided by amber PETE. This is due to amber PETE blocking light of wavelengths below 450 nm (UV and blue light range) and partially blocking the rest of the visible range (450 – 700 nm; green to red light). HDPE was the least effective. This is most likely due to high levels of oxygen (headspace) in the container, due to the different shape than PETE containers, as well as high oxygen transmission rates, resulting in higher rates of oxidation reactions. Clear PETE with UV block, which blocks wavelengths in the UV and small portion of the visible range (below 375 nm), did not perform as well as initially expected. Since oxidation of milk occurs at wavelengths below 500 nm (Cladman *et al.*, 1998), a different light blocking agent, which also block wavelengths in the visible range, might have been more suitable.

In general, different packaging materials do not influence lacks freshness off-flavor differently. According to this study, the preferred choice for packaging milk would thus be amber PETE. Clear PETE with UV block showed potential for protecting milk against oxidation, but not

substantially. The biggest disadvantage of using amber PETE would be that the container is not transparent, although the volume of milk in the container could still be observed.

Since PETE is more expensive than HDPE and shelf-life of freshly pasteurized milk is relatively short (18 - 21 days), PETE might be a more suitable and more economic packaging choice for extended shelf-life milk. Future work could thus be done on packaging of sterile milk with a 60 day shelf-life in PETE containers and incorporating light blocking agents that would block wavelengths in the visible range.

F. ACKNOWLEDGEMENTS

I would like to thank Eastman Chemical Co. not only for the financial support of this product, but also for supplying us with the HDPE and PETE bottles for this study, as well as their guidance and advice especially through Dr. Cheryl Heisey and Dr. Tom Clark.

I would also like to thank Walter Hartman, Kim Waterman and Harriet Williams for their time and support with all the various part of this study, as well as the many willing panelists that contributed to the sensory analysis of this project.

I would also like to thank my graduate committee, Dr. S.E. Duncan, Dr. J.E. Marcy, Dr. C.R. Hackney, and Dr. T. Long for their advice in their own areas of expertise, especially Dr. Duncan for her guidance and advice with this project.

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APPENDICES

APPENDIX A: Chocolate milk formula

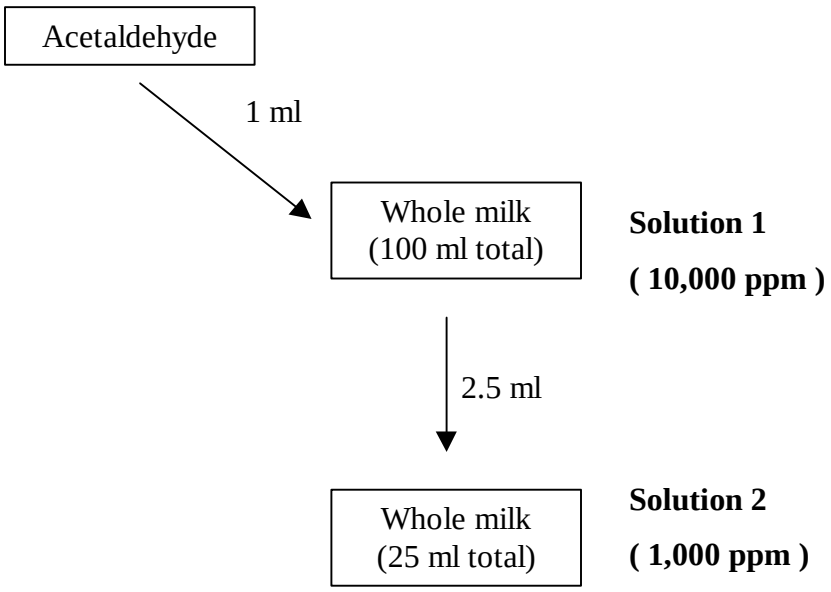
For 140 lbs of milk (3.25 % milkfat), blend and mix the following into warm (41 – 49^{o}C) milk.

- 8.89 lbs sugar (sucrose)
- 1.75 lbs cocoa
- 1.792 oz Chocoboost (control # 29943) (carragenan, standardized with dextrose)

Warm milk to a temperature between 41 and 48^{o}C. Mix the proper amounts of the dry ingredients together and then sprinkle, with agitation into the warm milk. Raise the temperature to pasteurize the mix. Homogenize while hot at 1500-2500 psi, then cool to 3^{o}C.

APPENDIX B: Dilution Chart for Spiking

Example: Whole milk



Concentration (ppb)	Volume of solution (ml)	Solution #	Total volume of milk (L)
200	0.2	2	1
400	0.4	2	1
800	0.8	2	1
1200	1.2	2	1
1600	0.16	1	1
3200	0.32	1	1
6400	0.64	1	1
9600	0.96	1	1
12800	1.28	1	1

APPENDIX C: Human Subjects Consent Form

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
INFORMED CONSENT FOR PARTICIPATION IN SENSORY EVALUATION

Title of Project: *Effect of Shelf-live and Light Exposure on Acetaldehyde Concentration in Milk Packaged in HDPE and PETE Bottles*

Principal Investigator: Dr. *Susan E. Duncan, Ph.D., R.D., Associate Professor*

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about milk.

The purpose of the project is to:

(i) establish the detection threshold of acetaldehyde in milk and a variety of milk products with different fat levels, and

(ii) determine the changes in acetaldehyde concentration in milk as related to storage time, light exposure and effect of different packaging materials used.

II. PROCEDURES

There will be __1__ session over a period of __1__ day involving about __15__ minutes at each session. You will be presented with approximately __30__ samples at each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide information that may be helpful to the determination of acetaldehyde threshold in milk and milk products. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

IV. EXTENT OF AN0NYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION

Please accept a small piece of candy, as a token of our appreciation for your participation in this project.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require sensory evaluation of milk and milk products.

_____

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address:_____

_____

Phone: _____

e-mail: _____

IX. SUBJECT'S PERMISSION (provide this portion for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study, which will involve sensory analysis of milk or milk products.

_____

Signature

Should I have any questions about this research or its conduct, I should contact:

Dr. Susan E. Duncan / (540) 231-8675

Investigator/Phone

Food Science and Technology / (540) 231-6806

Faculty/Phone

Dr. Tom Hurd / (540) 231-6077

Chair, IRB/Phone for Research Division

APPENDIX D: Scorecard for Sensory Evaluation of Acetaldehyde Threshold

SHELF-LIFE AND FLAVOR OF MILK

Name:_____ Replication __ for _____
Number:_____ Marleen van Aardt
Date: _____

Instructions:

Rinse your mouth with water before beginning. Expectorate the water into the container provided. You will be provided with a warm-up example to familiarize you with the expected taste of discrimination. Please complete the human subjects consent form while resting from the warm-up sample, to avoid adaptation of the palate. You will then receive ten sets of samples, each consisting of three samples. Two of the three samples in a set are the same and one is different. Please taste the samples in the order presented, from left to right. Circle the number of the sample in each set of three that tastes "different". Rinse your mouth with water between samples and expectorate all samples and the water. Rest between trays while eating a cracker to allow your palate to rest. Please close sample bottles after tasting, to avoid confusion between sample numbers.

First tray:
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____

Second tray:
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____

Third tray:
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____
Samples #: , , . Description of taste _____

Thank you very much for your participation in this study. Your participation in a next panel will be also be appreciated. You will be informed via e-mail of the date of the next session.

APPENDIX E: Sample preparation for training of panelists

Oxidation off-flavor

Score	*Treatment*
2 – trace, not sure	1 L of milk in glass container exposed to fluorescent lights for approximately 2 hours
4 – slight	1 L of milk in glass container exposed to fluorescent lights for approximately 8 hours
6 – moderate	1 L of milk in glass container exposed to fluorescent lights for approximately 24 hours
8 – strong	1 L of milk in glass container exposed to fluorescent light for approximately 2 – 3 days.

Acetaldehyde off-flavor

Score	*Treatment*
2 – trace, not sure	acetaldehyde concentration - approximately 500 ppb
4 – slight	acetaldehyde concentration - approximately 1,000 ppb
6 – moderate	acetaldehyde concentration - approximately 2,000 ppb
8 – strong	acetaldehyde concentration - approximately 4,000 ppb

Lacks freshness off-flavor

Since lacks off-flavor intensity was not consistent with code date, milks with various code dates were mixed to obtain desirable lacks freshness off-flavor levels.

APPENDIX F: Scorecard for Sensory Evaluation of Off-Flavor Determination

Name: _____ Replication _____

Date: _____

Instructions:

Please rinse your mouth with water and complete the human subjects consent form before beginning the evaluation. You will be provided with 10 samples. Each sample needs to be evaluated for light oxidized, acetaldehyde, and lacks freshness/not consumable off-flavors. Please mark your choice with an "X" in the appropriate blocks.

Thank you for your participation. Please check your schedule for the next sensory session.

SAMPLE # _____

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Light oxidized									
Acetaldehyde									
Lacks freshness									

SAMPLE # _____

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Light Oxidized									
Acetaldehyde									
Lacks freshness									

1- not noticeable; 2 – trace, not sure; 3 – faint; 4 – slight; 5 – mild; 6 – moderate; 7 – definite; 8 – strong; and 9 – very strong

* The scorecard given to panelists provided space for 10 samples.

APPENDIX G: Bacterial Counts (CFU/ml) for various containers over time

Packaging material	Count (log CFU/ml) at storage time			
	Psyhrotrophic		Std Plate Counts	
	0 d	18 d	0 d	18 d
Replication 1				
glass: exposed 1	<1.0	6.2	<1.0	5.2
glass: exposed 2	<1.0	4.7	0.5	4.9
glass: shielded 1	0.5	4.5	0.7	4.3
glass: shielded 2	0.8	7	0.8	6.4
HDPE: exposed 1	<1.0	4.2	<1.0	5
HDPE: exposed 2	<1.0	4	0.5	5.6
HDPE: shielded 1	<1.0	5.6	<1.0	5.5
HDPE: shielded 2	1	7.4	1.1	7.2
PETE (amber): exposed 1	<1.0	7.8	0.3	7.8
PETE (amber): exposed 2	0.5	5	0.5	5.3
PETE (amber): shielded 1	<1.0	6.2	<1.0	5.7
PETE (amber): shielded 2	<1.0	5.3	<1.0	6.2
PETE (clear): exposed 1	<1.0	7.2	<1.0	7.8
PETE (clear): exposed 2	<1.0	7.6	0.7	7.6
PETE (clear): shielded 1	<1.0	4.7	<1.0	5.3
PETE (clear): shielded 2	0.6	5.4	1.1	5.7
PETE (UV-block): exposed 1	<1.0	7.1	<1.0	7.2
PETE (UV-block): exposed 2	<1.0	7.3	<1.0	7.9
PETE (UV-block): shielded 1	<1.0	7.7	0.3	7.5
PETE (UV-block): shielded 2	0.3	7.2	0.8	7.6
Replication 2				
glass: exposed 1	<1.0	6.6	<1.0	5.7
glass: exposed 2	<1.0	7.2	<1.0	7.0
glass: shielded 1	0.6	6.7	1.1	6.8
glass: shielded 2	0.7	7.2	1.4	7.2
HDPE: exposed 1	<1.0	5.7	<1.0	5.7
HDPE: exposed 2	<1.0	7.0	<1.0	7.1
HDPE: shielded 1	1.2	7.2	1.2	7.0
HDPE: shielded 2	1.0	7.0	1.1	6.9
PETE (amber): exposed 1	0.5	7.0	<1.0	7.6
PETE (amber): exposed 2	<1.0	6.9	<1.0	7.2
PETE (amber): shielded 1	0.8	7.1	1.1	6.9
PETE (amber): shielded 2	1.4	7.8	1.0	7.3
PETE (clear): exposed 1	<1.0	5.7	<1.0	5.7
PETE (clear): exposed 2	<1.0	5.3	<1.0	5.7
PETE (clear): shielded 1	<1.0	7.2	0.9	7.3
PETE (clear): shielded 2	0.6	7.6	0.9	7.0

PETE (UV-block): exposed 1	<1.0	7.1	1.0	6.7
PETE (UV-block): exposed 2	<1.0	5.0	<1.0	5.3
PETE (UV-block): shielded 1	1.4	7.6	0.8	7.8
PETE (UV-block): shielded 2	<1.0	7.3	1.0	7.6

Replication 3

glass: exposed 1	0.8	6.3	0.3	7.0
glass: exposed 2	<1.0	5.5	<1.0	6.1
glass: shielded 1	0.9	6.5	<1.0	7.8
glass: shielded 2	<1.0	7.9	<1.0	7.9
HDPE: exposed 1	<1.0	6.2	<1.0	6.5
HDPE: exposed 2	<1.0	6.2	<1.0	6.7
HDPE: shielded 1	1.2	6.5	0.3	6.6
HDPE: shielded 2	<1.0	7.9	<1.0	7.8
PETE (amber): exposed 1	<1.0	7.0	<1.0	7.7
PETE (amber): exposed 2	0.6	7.7	<1.0	7.8
PETE (amber): shielded 1	1.2	7.9	0.5	7.8
PETE (amber): shielded 2	<1.0	5.9	<1.0	7.7
PETE (clear): exposed 1	<1.0	7.2	<1.0	7.8
PETE (clear): exposed 2	<1.0	6.0	<1.0	6.3
PETE (clear): shielded 1	<1.0	6.7	<1.0	7.7
PETE (clear): shielded 2	<1.0	7.9	<1.0	7.6
PETE (UV-block): exposed 1	<1.0	5.0	0.3	5.3
PETE (UV-block): exposed 2	1.2	6.3	0.8	5.6
PETE (UV-block): shielded 1	0.5	7.9	0.6	7.8
PETE (UV-block): shielded 2	<1.0	6.5	<1.0	6.2

*** All Coliform counts done on day 0 were <1.0 log CFU/ml**

Appendix H

Human threshold for acetaldehyde in nonfat (0.5% milkfat) milk

Replicate 1

Subject	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**200**	**400**	**800**	**1200**	**1600**	**3200**	**6400**	**9600**	**12800**	**17100**	**21400**	**thresh.**
1	0	1	1	1	1	1	1	1	*	1			14
2	0	0	1	0	0	0	0	1	*	1			4525
3	0	0	0	1	1	0	1	1	*	1			2263
4	0	0	0	0	0	0	0	1	*	1			4525
5	0	1	0	1	0	0	0	0	*	0	1	0	23406
6	0	1	0	0	0	1	0	0	*	1			18102
7	0	0	1	1	1	0	1	1	*	1			2263
8	0	1	0	1	0	0	0	1	*	0	0	1	23406
9	0	0	0	1	0	0	1	0	*	1			18102
10	0	1	1	0	1	1	1	1	*	1			980
11	0	0	1	0	1	1	1	1	*	1			980
12	0	0	0	1	0	1	0	1	*	1			4525
13	0	1	1	0	1	1	1	1	*	1			980
14	0	1	0	0	1	1	0	0	*	1			18102
15	0	0	0	1	0	0	0	0	*	1			18102
16	0	0	1	1	0	1	1	1	*	1			1386
17	0	0	0	0	1	0	1	0	*	1			18102
18	0	0	1	0	1	1	0	1	*	1			4525
19	0	0	0	1	1	1	1	0	*	1			18102
20	0	0	1	1	1	1	1	1	*	1			283
21	0	1	1	1	1	0	0	0	*	1			18102
22	0	0	0	0	0	1	0	1	*	0	1	1	14795
23	0	0	0	1	0	1	0	1	*	1			4525
24	0	0	0	1	1	1	1	1	*	1			566
25	0	1	0	0	1	1	1	1	*	1			980

* Level 9600 ppb was not spiked for this duplicate — **Group threshold: 3685**

0 - incorrect response

1 - correct response

Replicate 2

Subject	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**200**	**400**	**800**	**1200**	**1600**	**3200**	**6400**	**9600**	**12800**	**17100**	**21400**	**thresh.**
1	1	1	0	0	1	1	0	1	1	1			4525
2	0	1	0	1	1	0	0	1	1	0	1	1	14795
3	0	1	0	1	1	0	1	1	1	1			2263
4	0	1	1	0	1	1	1	1	1	1			980
5	0	0	0	1	1	1	1	1	1	1			566
6	0	0	1	0	0	0	1	1	0	0	1	1	14795
7	0	0	0	0	0	0	0	1	1	0	0	1	23406
8	0	0	1	0	0	1	0	0	1	1			7838
9	0	1	0	0	1	0	0	0	0	0	0	0	23406
10	0	1	0	0	1	0	0	1	0	1	1	1	11085
11	0	0	0	0	0	1	0	1	1	1			4525
12	0	0	1	0	1	1	0	1	0	1	1	0	23406
13	0	0	0	1	1	0	1	1	1	1			2263
14	0	0	1	1	1	1	1	1	1	1			283
15	0	0	1	1	1	0	1	1	1	1			2263
16	0	0	0	1	1	1	1	1	1	1			566
17	0	1	0	1	0	1	0	0	1	0			18102
18	0	0	1	1	1	1	1	1	1	1			283
19	0	1	0	1	1	1	1	1	1	0	1	1	14795
20	0	0	0	0	0	0	1	0	0	0	0	1	23406
21	0	0	1	0	1	0	0	1	1	1			4525
22	0	1	1	1	1	1	1	1	1	1			14
23	0	0	1	1	0	1	0	1	0	1	0	1	23406
24	0	0	1	1	1	1	1	1	1	0			18102
25	0	1	1	0	1	1	0	1	1	0	1	1	14795

0 - incorrect response — **Group threshold: 4193**

1 - correct response

Ave between duplications: 3939

Human threshold for acetaldehyde in low fat (2% milkfat) milk

Replicate 1

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**200**	**400**	**800**	**1200**	**1600**	**3200**	**6400**	**9600**	**12800**	**17100**	**21400**	**thresh.**
1	0	0	0	0	1	0	0	0	1	1			7838
2	0	0	1	0	1	0	0	0	1	1			7838
4	0	0	0	0	0	1	0	1	1	1			4525
5	0	0	0	1	1	1	1	1	1	1			566
6	0	1	1	0	1	1	1	1	1	1			980
8	0	1	1	0	1	1	0	0	0	0	1	0	23406
9	0	0	0	1	0	1	1	1	1	1			1386
10	0	0	0	1	1	1	1	1	1	1			566
11	0	1	1	0	0	1	1	1	1	1			1386
12	0	0	0	0	0	0	0	1	1	1			4525
13	0	0	1	0	1	1	1	1	1	1			980
14	0	1	0	0	1	0	0	0	0	0	0	0	23406
15	0	0	0	0	1	0	1	1	1	1			2263
16	0	1	0	1	1	1	1	1	1	1			566
17	0	0	0	0	1	0	0	1	0	1	1	0	23406
18	0	1	0	0	1	0	1	0	1	1			7838
19	0	0	1	0	1	1	0	1	0	1	1	1	11085
20	0	0	1	1	0	1	1	1	1	1			1386
21	0	0	0	0	1	0	0	0	1	0	1	1	14795
22	0	0	0	0	0	0	0	1	0	0	0	0	23406
23	0	1	1	1	1	0	0	1	1	1			4525
24	0	0	0	1	0	0	0	1	0	1	1	0	23406
25	0	0	0	1	0	0	1	1	1	1			2263

0 - incorrect response
1 - correct response

Group threshold: 4096

Replicate 2

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**200**	**400**	**800**	**1200**	**1600**	**3200**	**6400**	**9600**	**12800**	**17100**	**21400**	**thresh.**
1	0	1	1	1	0	0	1	1	0	1	0	0	23406
2	0	0	1	1	1	1	1	1	1	1			283
3	0	0	0	0	1	1	1	0	1	1			7838
4	0	1	1	1	1	1	1	1	1	1			18102
5	0	1	1	0	0	1	1	0	1	1			7838
6	0	1	1	0	0	0	1	1	1	0	1	1	14795
7	0	0	1	1	1	1	1	1	1	1			283
8	0	0	1	0	0	1	1	1	1	1			1386
9	0	0	0	1	1	1	0	1	1	0			23406
10	0	0	1	0	1	1	0	0	0	1	1	0	23406
11	0	0	1	1	1	1	1	1	1	1			283
12	0	0	0	0	0	1	0	1	1	1			4525
13	0	1	1	1	1	1	1	1	1	1			18102
14	0	1	0	0	0	0	0	1	0	0	0	0	23406
15	0	0	1	0	1	1	1	1	1	1			980
16	0	0	0	0	1	1	1	1	1	1			980
17	0	0	0	0	1	1	1	1	1	1			980
18	0	1	0	1	1	0	1	1	1	1			2263
19	0	1	0	1	1	1	1	1	1	1			566
20	0	0	1	0	1	1	1	1	1	1			980
21	0	0	0	0	1	0	1	0	0	1	1	1	11085
22	0	1	1	1	1	1	1	1	1	1			18102
23	0	1	1	1	1	1	1	1	1	1			14795
24	0	1	0	1	0	1	1	0	1	1			7838
25	0	1	0	1	1	0	1	1	1	1			2263

0 - incorrect response
1 - correct response

Group threshold: 3943

Ave between duplications: 4020

Human threshold for acetaldehyde in whole milk (3.25% milkfat)

Replicate 1

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**200**	**400**	**800**	**1200**	**1600**	**3200**	**6400**	**9600**	**12800**	**17100**	**21400**	**thresh.**
1	0	0	1	1	1	1	0	1	1	0	1	1	14795
2	0	0	1	0	1	0	0	0	0	1	0	0	23406
3	0	0	1	1	1	1	1	1	1	1			283
4	0	0	0	1	0	0	0	0	1	1			7838
5	0	0	0	0	0	1	1	1	1	1			1386
6	0	0	0	1	1	0	1	0	1	1			7838
7	0	0	0	0	1	0	1	1	1	0	0	1	23406
8	0	0	1	1	0	0	1	1	1	1			2263
9	0	0	1	0	0	0	1	1	1	0	0	1	23406
10	0	1	0	1	1	0	1	1	1	0	1	1	14795
11	0	0	1	0	1	1	1	1	1	1			980
12	0	1	0	1	0	0	0	0	1	0	0	1	23406
13	0	0	1	1	1	1	1	1	1	0	1	1	14795
14	0	0	0	0	1	1	0	1	1	1			4525
15	0	1	1	0	1	1	1	1	1	1			980
16	0	1	1	0	0	0	1	1	1	0	0	1	23406
17	0	1	0	1	0	1	1	1	1	1			1386
18	0	1	0	0	1	1	1	1	1	1			980
19	0	1	0	1	1	1	1	0	1	1			7838
20	0	0	1	1	1	1	1	1	1	1			283
21	0	0	1	0	1	1	1	1	1	0	1	1	14795
22	0	1	1	1	1	1	1	1	1	1			14
23	0	0	1	1	1	0	1	1	0	0	1	0	23406
24	0	0	0	1	1	1	1	1	1	1			566
25	0	0	0	0	0	0	1	0	1	0	0	0	23406

0 - incorrect response
1 - correct response

Group threshold: 3989

Replicate 2

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**200**	**400**	**800**	**1200**	**1600**	**3200**	**6400**	**9600**	**12800**	**17100**	**21400**	**thresh.**
1	0	0	0	0	0	1	1	0	1	1			7838
2	0	1	1	1	1	1	1	1	0	1	1	1	11085
3	0	0	0	0	1	1	1	1	1	1			980
4	0	1	1	1	1	1	1	1	1	0	0	1	23406
5	0	1	1	1	1	1	1	1	1	0	1	1	14795
6	0	0	0	0	1	1	1	1	1	1			980
7	0	0	1	0	0	0	0	0	0	1	1	1	11085
8	0	1	0	1	1	0	1	0	1	1			7838
9	0	1	1	1	1	1	0	1	0	1	1	1	11085
10	0	0	1	1	1	1	1	1	0	1	1	1	11085
11	0	0	1	0	1	0	1	1	1	1			2263
12	0	1	0	1	0	0	1	1	1	1			2263
13	0	0	0	1	1	1	0	1	1	1			4525
14	0	1	0	0	1	0	0	1	1	1			4525
15	0	1	0	1	1	0	1	1	1	1			2263
16	0	1	1	1	1	1	1	1	1	1			14
17	0	1	0	0	1	0	0	1	0	1	1	1	11085
18	0	1	0	0	1	0	1	0	0	0	0	1	23406
19	0	1	0	1	0	1	0	0	1	0	1	1	14795
20	0	0	1	0	1	1	1	1	1	1			980
21	0	0	0	0	1	0	0	1	1	1			4525
22	0	1	0	0	0	1	1	1	0	1	0	1	23406
23	0	1	1	1	1	1	1	1	1	1			14
24	0	0	0	1	1	1	1	0	1	0	1	1	14795
25	0	1	1	0	1	0	1	1	0	1	0	1	23406

0 - incorrect response
1 - correct response

Group threshold: 4091

Ave between duplications: 4040

Human threshold for acetaldehyde in chocolate milk

Replicate 1

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**400**	**800**	**1600**	**3200**	**6400**	**9600**	***12800***	**19200**	**25600**	**34100**	**42700**	**thresh.**
1	0	0	1	0	0	1	1	*1*	0	1	1	1	22170
2	0	0	0	1	1	1	1	*0*	1	1			1131
3	0	0	1	1	0	1	1	*1*	1	1			4525
4	0	0	0	0	0	1	1	*1*	1	1			4525
5	0	0	0	0	0	0	0	*0*	0	0	0	0	46757
6	0	0	0	0	0	1	0	*0*	1	1			13576
7	0	0	1	0	0	0	0	*0*	1	1			13576
8	0	0	0	1	0	1	1	*1*	0	1	0	1	46757
9	0	1	0	0	0	1	1	*1*	0	0	1	1	29546
10	0	0	0	0	1	1	0	*0*	0	1	1	1	22170
11	0	1	0	0	0	0	1	*0*	1	1			7838
12	0	0	1	1	1	1	1	*0*	1	1			566
13	0	0	0	1	1	1	1	*0*	0	1	1	1	22170
14	0	0	0	1	1	1	1	*0*	1	1			1131
15	0	0	0	1	1	1	1	*1*	1	1			1131
16	0	1	1	1	1	1	0	*1*	1	1			13576
17	0	0	1	0	0	0	0	*0*	1	1			13576
18	0	1	0	1	1	0	0	*0*	1	1			13576
19	0	1	0	0	0	0	1	*0*	0	1	1	0	46757
20	0	0	0	0	0	1	1	*0*	1	0	0	1	46757
21	0	0	0	0	1	1	1	*0*	1	1			2263
22	0	1	1	0	1	1	1	*0*	1	1			2263
23	0	0	0	0	1	1	1	*0*	0	1	1	1	22170
24	0	0	0	0	1	1	0	*1*	0	1	0	0	46757
25	0	1	1	0	0	0	0	*1*	0	0	1	1	29546

Level 12800 ppb is eliminated from this series due to incorrect spiking

0 - incorrect response **Group threshold:** **10154**

1 - correct response

Replicate 2

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**400**	**800**	**1600**	**3200**	**6400**	**9600**	**12800**	**19200**	**25600**	**34100**	**42700**	**thresh.**
1	0	1	0	1	1	1	1	1	1	1			1131
2	0	1	0	0	1	1	1	1	1	1			2263
3	0	0	0	1	1	1	0	1	1	1			11085
4	0	1	0	1	1	1	1	1	1	1			1131
5	0	1	1	1	0	1	1	1	1	1			4525
6	0	0	1	0	0	1	1	0	1	1			15677
7	0	0	0	0	0	1	1	1	1	1			4525
8	0	1	0	1	1	0	1	0	1	1			15677
9	0	1	0	0	1	0	0	0	0	1	0	0	46757
10	0	1	0	0	0	1	0	0	0	0	0	1	46757
11	0	1	0	1	1	0	0	0	1	0	1	1	29546
12	0	0	0	1	1	1	1	1	1	1			1131
13	0	1	0	0	1	1	1	0	1	1			15677
14	0	0	0	0	1	1	1	1	1	1			2263
15	0	1	0	1	1	0	0	0	1	1			15677
16	0	0	0	1	0	0	0	1	0	0	1	1	29546
17	0	0	0	0	1	0	0	0	1	0	0	0	46757
18	0	1	0	1	1	1	1	1	1	1			1131
19	0	0	0	0	0	0	1	1	0	1			36204
20	0	0	1	1	1	1	1	1	1	1			566
21	0	0	0	0	1	0	0	0	1	0	0	1	46757
22	0	0	0	0	0	1	0	1	0	0	1	0	46757
23	0	1	1	0	1	1	0	1	1	1			11085
24	0	0	0	0	1	1	0	0	1	0			36204
25	0	0	1	0	1	1	1	0	0	1	1	0	46757

0 - incorrect response **Group threshold:** **9941**

1 - correct response

Ave between duplications: **10048**

Human threshold for acetaldehyde in spring water

Replicate 1

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**2.5**	**5**	**10**	**20**	**40**	**80**	**120**	**160**	**320**	**480**	**640**	**thresh.**
1	0	1	0	0	0	1	1	1	1	1			28
2	0	1	0	0	1	0	0	0	0	1	1	1	226
3	0	1	1	0	1	0	0	1	0	1	0	1	784
4	0	0	0	0	1	1	1	1	1	1			14
5	0	0	0	1	1	1	1	1	1	1			7
6	0	0	1	0	0	0	0	1	1	1			98
7	0	1	1	0	0	0	0	0	1	0	1	1	392
8	0	0	0	0	0	1	1	1	1	1			28
9	0	0	0	1	0	1	0	0	0	0	1	1	392
10	0	0	0	1	0	1	1	1	1	1			28
11	0	0	0	1	0	0	0	0	1	1			139
12	0	0	0	0	0	1	0	1	0	1	0	0	784
13	0	0	1	0	1	1	0	1	1	1			98
14	0	0	1	1	1	0	1	1	1	1			57
15	0	0	1	0	0	1	0	1	0	0	1	0	784
16	0	0	0	0	0	0	0	0	0	1	0	0	784
17	0	0	0	0	0	0	0	0	0	0	0	0	784
18	0	1	0	0	1	0	1	0	1	0	1	1	392
19	0	0	0	0	0	0	1	0	0	0	0	1	784
20	0	0	0	1	0	0	0	0	1	1			139
21	0	1	0	1	1	0	1	1	1	1			57
22	0	1	0	1	1	1	1	1	1	1			7
23	0	1	1	0	0	0	1	0	1	0	1	1	392
24	0	0	0	0	0	0	1	1	0	0	0	1	784
25	0	0	1	0	1	0	0	0	0	0	1	0	784

0 - incorrect response
1 - correct response

Group threshold: 156

Replicate 2

Person	Concentration (ppb)										*Retest*	*Retest*	Ind.
	0	**2.5**	**5**	**10**	**20**	**40**	**80**	**120**	**160**	**320**	**480**	**640**	**thresh.**
1	0	1	0	1	0	0	0	0	1	1			139
2	0	1	0	1	0	0	0	0	1	0	1	1	392
3	0	1	0	1	1	0	1	1	0	1	1	1	226
4	0	0	0	0	0	0	1	1	0	1	0	0	784
5	0	0	1	1	0	0	0	1	1	1			98
6	0	0	0	0	0	0	1	0	1	0	1	1	392
7	0	1	1	0	0	0	0	0	1	1			139
8	0	0	1	1	1	1	0	0	1	1			139
9	0	0	0	0	1	0	1	1	1	1			57
10	0	0	0	1	1	0	1	1	0	0	1	1	392
11	0	0	1	0	1	1	1	1	1	1			14
12	0	0	1	0	1	1	1	1	1	1			14
13	0	0	0	0	0	1	0	0	0	1	1	1	226
14	0	0	1	0	0	0	0	0	1	1			139
15	0	0	1	0	0	0	1	0	0	0	1	0	784
16	0	0	0	0	0	0	0	1	1	1			98
17	0	1	0	0	0	0	0	0	0	0	0	1	784
18	0	0	0	0	1	0	1	0	0	1	0	1	784
19	0	0	1	0	0	0	0	1	1	1			98
20	0	1	1	0	1	1	0	0	0	1	1	1	226
21	0	0	0	0	0	1	0	0	1	1			139
22	0	0	0	1	0	1	0	1	1	1			98
23	0	0	0	0	0	0	1	0	1	1			139
24	0	1	0	0	0	0	1	1	0	0	0	1	784
25	0	1	1	1	0	1	0	1	1	1			98

0 - incorrect response
1 - correct response

Group threshold: 177

Ave between duplications: 167