

Available online at www.sciencedirect.com



Food Chemistry 87 (2004) 1-9

Food Chemistry

www.elsevier.com/locate/foodchem

# Shelf life of whole pasteurized milk in Greece: effect of packaging material

Panagiota Zygoura <sup>a</sup>, Theodora Moyssiadi <sup>a</sup>, Anastasia Badeka <sup>a</sup>, Efthymia Kondyli <sup>b</sup>, Ioannis Savvaidis <sup>a</sup>, Michael G. Kontominas <sup>a,\*</sup>

<sup>a</sup> Laboratory of Food Chemistry and Technology, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece <sup>b</sup> National Agricultural Research Foundation, Dairy Research Institute, Ioannina 45216, Greece

Received 17 June 2003; received in revised form 15 October 2003; accepted 15 October 2003

# Abstract

Chemical, microbiological and sensorial changes in whole pasteurized milk stored under fluorescent light at 4 °C in 500 ml bottles, made of: (a) multilayer pigmented [HDPE + 2% TiO<sub>2</sub>/HDPE + 4% carbon black/HDPE + 2% TiO<sub>2</sub>], 550–600  $\mu$ m in thickness, (b) monolayer pigmented [HDPE + 2% TiO<sub>2</sub>], 550–600  $\mu$ m in thickness, (c) clear PET, 300–350  $\mu$ m in thickness and (d) pigmented [PET + 2% TiO<sub>2</sub>], 300–350  $\mu$ m in thickness, were monitored for a period of 7 days. Milk packaged in coated paperboard cartons and stored under the same experimental conditions served as the "control" sample. Data were obtained for lipid oxidation, lipolysis, proteolysis, vitamin A and riboflavin contents, microbial growth including mesophilic and psychrotrophic counts and sensorial attributes (odour and taste) of whole pasteurized milk. Results showed satisfactory protection of milk packaged in all packaging materials with regard to microbiological and chemical parameters assessed over the 7 day period. Vitamin A losses, recorded after 7 days of storage, were, respectively, 8.8%, 10.5%, 29.8%, 50.9% and 14.0% in samples packaged in multilayer HDPE, monolayer HDPE, pigmented PET, clear PET bottles and control samples. Respective losses of riboflavin were 18.4%, 20.6%, 30.9%, 47.1% and 19.8%. Based on organoleptic analysis, the shelf life of whole pasteurized milk in Greece is 5 days. The best overall protection to the product was provided by the multilayer and monolayer pigmented HDPE bottles at a thickness of 550–600  $\mu$ m. Such packaging materials are currently finding their way into the fresh milk packaging market. © 2003 Elsevier Ltd. All rights reserved.

Keywords: Milk; Plastic packaging; Quality retention

# 1. Introduction

The main factors affecting the keeping quality of pasteurized milk are raw milk quality, severity of heat treatment, post pasteurization contamination and storage temperature (IDF, 1986). Packaging is also a factor of utmost importance, effectively protecting the product from microbial recontamination, light and oxygen (Erickson, 1997;Ravanis & Lewis, 1995; Skibsted, 2000; Vassila, Badeka, Kondyli, Savvaidis, & Kontominas, 2002).

Milk quality deterioration is perceived by the consumer through off-flavours that may be caused by chemical, physicochemical or microbiological changes in the product (Allen & Joseph, 1985; Rysstad, Ebbesey, & Eggestad, 1998; Thomas, 1981; Valero, Villamiel, Sanz, & Martinez-Castro, 2000; van Aardt, Duncan, Marcy, Long, & Hackey, 2001). Among these defects, light-induced off-flavours are probably the most common in milk, attributed to two distinct causes. The first, a "burnt sunlight flavour", develops during the first 2 or 3 days of storage and is caused by degradation of sulfurcontaining amino acids of the whey proteins (Marsili, 1999). The second is a metallic or cardboardy off-flavour (lack of freshness) that develops two days later and does not dissipate. This off-flavour is attributed to lipid oxidation (Barnard, 1972). Light exposure, especially at wavelengths below 500 nm, also causes destruction of

Abbreviations: HDPE = High density polyethylene; PET = Polyethylene terephthalate.

<sup>\*</sup> Corresponding author. Tel.: +30-265-10-98-342; fax: +30-265-10-98-795.

E-mail address: mkontomi@cc.uoi.gr (M.G. Kontominas).

light-sensitive vitamins, mainly vitamin A and riboflavin (Fanelli, Burlew, & Gabriel, 1985; Sattar, de Man, & Alexander, 1977).

Packaging can directly influence the development of light-induced flavour by protecting the product from both light and oxygen (Schröder, 1982; Skibsted, 2000; Vassila et al., 2002). Apart from traditional glass bottles and coated paperboard cartons, all-plastic containers, namely: high density polyethylene, polycarbonate and polyethylene terephthalate, have been used in pasteurized milk packaging (Defosse, 2000; Schröder, Scott, Bland, & Bishop, 1985). Problems with all-plastic containers, used in numerous studies mentioned above, include transmission of light and permeability to oxygen. It should be noted, however, that oxidative reactions were reported to take place in milk packaged, even in coated paperboard cartons, which were found to be more or less permeable to oxygen (Rysstad et al., 1998; Schröder et al., 1985). More recently, PET and coextruded HDPE bottles have been used for fresh milk packaging (Bakish & Hatfield, 1997; Cladman, Scheffer, Goodrich, & Griffiths, 1998; van Aardt et al., 2001). PET has excellent mechanical properties, a good barrier to  $O_2$  and reduces the adverse effects of light on milk quality in the form of pigmented bottles. On the other hand, pigmented HDPE bottles, both monolayer and multilayer, with a greater thickness than PET, are finding their way into the fresh milk packaging market. Both provide excellent convenience and protection through easy opening and reclosing, thus minimizing recontamination (Cladman et al., 1998).

The objective of this study was to evaluate chemical, microbiological and sensorial stabilities of whole milk packaged in PET and HDPE bottles with different light and oxygen barriers, as compared to that provided by the coated paperboard carton under actual commercial conditions of storage.

# 2. Materials and methods

#### 2.1. Packaging materials

Four different packages were evaluated with regard to their potential effect on milk quality during refrigerated storage. The four packages included: (a) a 1/2 l pigmented [HDPE + 2% TiO<sub>2</sub>/HDPE + 4% carbon black pigment/HDPE + 2% TiO<sub>2</sub>] 3 layer coextruded bottle, 550–600  $\mu$ m in thickness, (b) a 1/2 litre pigmented [HDPE + 2% TiO<sub>2</sub>] bottle, 550–600  $\mu$ m in thickness, (c) a 1/2 litre clear PET bottle, 300–350  $\mu$ m in thickness and (d) a 1/2 litre pigmented [PET + 2% TiO<sub>2</sub>] bottle, 300– 350  $\mu$ m in thickness. The white (TiO<sub>2</sub>) pigmented bottles were chosen on the basis of consumers' first preference (White, 1985) with regard to colour and their potential protective effect against harmful incident light. The carbon black pigment has been suggested (Schröder, 1982) for maximum protection against UV–Vis radiation. Both pigments are FDA approved. Bottle (a), containing inner and outer white coloured layers, was white in colour. One half litre coated paperboard cartons served as the commercial "control" samples. Experimental HDPE and PET bottle samples were produced by ARGO Ltd. on a three extruder coextrusion line (Soplar SA, Switzerland) and an injection stretch blow moulding line (Nissei ASB, USA), respectively; coated paperboard cartons were provided by DODONI Ltd.

#### 2.2. Sample preparation and handling

Homogenized whole milk (3.5% fat) was obtained from a local dairy plant (DODONI Ltd) immediately after pasteurization. The milk was aseptically dispensed into the sterilized bottles and the coated paperboard cartons at the plant laboratory. Bottles were sealed using polypropylene screw caps, while coated paperboard cartons were sealed on the production line sealer. Headspace for both cartons and bottles was between 25 and 40 ml. The filled containers were transported to the laboratory within one hour in polystyrene foam ice boxes packed in ice. Bottles were stored at  $4 \pm 0.5$  °C for a period of up to 7 days under perpendicular fluorescent light provided by one 55 W cool white fluorescent lamp in a commercial display cabinet. The fluorescent lamp produced  $825 \pm 50$  lux on the side surface of the containers, measured with a GE light meter (General Electric Co, USA). Milk samples (100 ml) were collected from sealed bottles at sampling times of: 1, 3, 5 and 7 days after initial packaging, for chemical, microbiological and sensorial testing. Testing at day 0 was carried out on milk samples immediately after packaging. At each sampling day, six different samples (each from a different container) were assayed (n = 6). After sampling, each container was discarded.

#### 2.3. Chemical tests

#### 2.3.1. Protein hydrolysis assay

The protein hydrolysis assay was carried out spectrophotometrically, according to the method of Hull (1947) and expressed as mg free tyrosine/ml of milk.

#### 2.3.2. Lipolysis

The lipolysis assay was carried out volumetrically, according to the method of Deeth, Fitzgerald, and Wood (1975) and expressed as  $\mu$  equiv FFA/ml of milk.

#### 2.3.3. Lipid oxidation

Lipid oxidation was evaluated spectrophotometrically using the TBA test as described by King (1962).

#### 2.3.4. Determination of vitamins

Vitamin A was determined using the HPLC method of Zahar and Smith (1990). Riboflavin was determined by the HPLC method of Toyosaki, Yamamoto, and Mineshita (1988).

# 2.4. Physical tests

# 2.4.1. Light transmission testing

Spectral transmission characteristics over the wavelength range of 350–780 nm (Rysstad et al., 1998; van Aardt et al., 2001), for all packaging materials, were measured with a Shimadzu model 2100 UV–Vis recording spectrophotometer.

#### 2.4.2. Headspace oxygen measurement

A rubber septum (Systech Instr. Ltd., UK) was glued onto the surface of the bottle and pierced with a 23 gauge needle connected to a precalibrated headspace analyzer model Gaspace 2 (Systech Instr. Ltd., UK) giving a direct reading of the % oxygen in the bottle headspace.

# 2.4.3. Determination of oxygen transmission rates

Oxygen transmission rates for all packages (bottles and cartons) were measured using the Oxtran 2/20 oxygen permeability tester (Mocon Controls, USA) at RH = 60%, T = 22 °C and were expressed as ml/package·day·atm.

#### 2.5. Microbiological tests

# 2.5.1. Mesophilic counts

To monitor possible microbial post-pasteurization contamination of milk in the various containers, standard plate counts were determined according to the IDF standard No. 100 B (IDF, 1991a).

## 2.5.2. Psychrotrophic counts

Psychrotrophic counts were determined according to the IDF standard No. 132 A (IDF, 1991b).

## 2.6. Sensory evaluation

A panel of 17 individuals, consisting of faculty and graduate students, members of the Food Chemistry and Technology Laboratory in the Department of Chemistry, were trained to differentiate between burnt (light oxidized) flavour and stale (lack of freshness) flavour of milk samples. Sensory data were collected between days 0 and 7 of storage. Milk samples (30 ml) were presented to panellists in individual sensory booths as sets of three or four with a resting interval of 1 or 2 min between sample sets. Panellists rated each sample for intensity and type of off-flavour on a scale from 0–5, where a score of 5 corresponded to very good flavour milk and 0

to unfit for consumption (very strong off-flavour) milk (IDF, 1987).

#### 2.7. Statistical analysis

Data were subjected to analysis of variance using the Excel 97 software programme (Microsoft, CA, USA) and, where statistical differences were noted, differences among packages were determined, using the least significant difference (LSD) test. Significance was defined at P < 0.05.

## 3. Results and discussion

# 3.1. Microbiological assessment of milk quality

#### 3.1.1. Mesophilic counts

Mesophilic counts of milk stored in different packaging materials, as a function of storage time, are shown in Table 1. Initial (day 0) counts equal to 4.65 log cfu/ml were high, indicative of the poor milk collection practices throughout Greece. Such high microbial loads are responsible for the short shelf life of domestic pasteurized milk which does not normally exceed 5 days. No statistically significant (P > 0.05) differences in mesophilic counts were recorded for milk samples in all packaging materials for a given sampling day during the entire 7 day storage period. After 7 days of storage, mesophilic counts were between 6.56 and 7.16 log cfu/ ml, values very close to the upper limit ( $10^6-10^7$  cfu/ml) for keeping quality of milk (IDF, 1986).

Likewise, Erickson (1997) reported no statistically significant differences (P > 0.05) in total counts between HDPE jugs (5.38 log cfu/ml) and paperboard cartons (5.47 log cfu/ml) after 8 days of milk storage (5 °C) but statistically significant differences in total counts between clear (3.22 log cfu/ml) and opaque (1.50 log cfu/ml) HDPE pouches under the same experimental conditions. Vassila et al. (2002) also reported no significant differences for milk total counts packaged in different pouch materials (6.01–6.34 log cfu/ml) and paperboard cartons (6.49 log cfu/ml).

#### 3.1.2. Psychrotrophic counts

Psychrotrophic counts of milk stored in different packaging materials, as a function of storage time, are shown in Table 2. No statistically significant (P > 0.05) differences in phychrotrophic counts were recorded for milk samples in all packaging materials for a given sampling day during the entire 7 day storage period. After 7 days of storage, relatively high psychrotrophic counts, between 6.11 and 6.69 log cfu/ml, were recorded for all packaging materials. Erickson (1997) reported a psychrotrophic count of 5.34 and 5.20 log cfu/ml for whole milk stored in paperboard cartons and HDPE

| Mesophilic counts of whole pasteurized milk packaged in various containers during storage at 4 °C |   |                   |                   |                   |                   |   |  |
|---|---|-------------------|-------------------|-------------------|-------------------|---|--|
| Packaging material  | Mesophilic counts (log cfu/ml)<br>Days of storage at 4 °C |                   |                   |                   |                   |   |  |
|   | 0   | 1                 | 3                 | 5                 | 7                 | _ |  |
| 3 layer pigmented, coextruded HDPE bottle   | 4.65 <sup>a</sup>   | 4.70 <sup>a</sup> | 4.76 <sup>a</sup> | 4.85 <sup>a</sup> | 7.04 <sup>a</sup> |   |  |
| Monolayer pigmented HDPE bottle   | 4.65 <sup>a</sup>   | 4.65 <sup>a</sup> | 4.67 <sup>a</sup> | 4.79 <sup>a</sup> | 7.16 <sup>a</sup> |   |  |
| Clear PET bottle  | 4.65 <sup>a</sup>   | 4.66 <sup>a</sup> | 4.58 <sup>a</sup> | 4.72 <sup>a</sup> | 6.56 <sup>a</sup> |   |  |
| Pigmented PET bottle  | 4.65 <sup>a</sup>   | 4.66 <sup>a</sup> | 4.68 <sup>a</sup> | 4.81 <sup>a</sup> | 6.64 <sup>a</sup> |   |  |
| Coated paperboard carton  | 4.65 <sup>a</sup>   | 4.69 <sup>a</sup> | 4.71 <sup>a</sup> | 5.15 <sup>a</sup> | 6.94 <sup>a</sup> |   |  |

Table 1 Mesophilic counts of whole pasteurized milk packaged in various containers during storage at 4 °C

Values reported are the means of six replicates (n = 6).

<sup>a</sup> Values within a column followed by different letters are significantly different (P < 0.05).

Table 2

Psychrotrophic counts of whole pasteurized milk packaged in various containers during storage at 4 °C

| Packaging material                        | Psychrotrophic counts (log cfu/ml)<br>Days of storage at 4 °C |                   |                   |                   |                   |  |
|---|---|-------------------|-------------------|-------------------|-------------------|--|
|   | 0   | 1                 | 3                 | 5                 | 7                 |  |
| 3 layer pigmented, coextruded HDPE bottle | 3.51 <sup>a</sup>   | 3.04 <sup>a</sup> | 3.58ª             | 4.40 <sup>a</sup> | 6.40 <sup>a</sup> |  |
| Monolayer pigmented HDPE bottle           | 3.51 <sup>a</sup>   | 3.15 <sup>a</sup> | 3.72 <sup>a</sup> | 4.48 <sup>a</sup> | 6.30 <sup>a</sup> |  |
| Clear PET bottle                          | 3.51 <sup>a</sup>   | 2.96 <sup>a</sup> | 3.36 <sup>a</sup> | 4.56 <sup>a</sup> | 6.69 <sup>a</sup> |  |
| Pigmented PET bottle                      | 3.51 <sup>a</sup>   | 2.83 <sup>a</sup> | 3.48 <sup>a</sup> | 4.89 <sup>a</sup> | 6.54 <sup>a</sup> |  |
| Coated paperboard carton                  | 3.51 <sup>a</sup>   | 2.98 <sup>a</sup> | 3.61 <sup>a</sup> | 4.92 <sup>a</sup> | 6.11 <sup>a</sup> |  |

Values reported are the means of six replicates (n = 6).

<sup>a</sup> Values within a column followed by different letters are significantly different (P < 0.05).

jugs, respectively, after 8 days of storage, while Cladman et al. (1998) reported psychrotrophic counts of 3.23 and 4.52 log cfu/ml for 2% milk packaged in PET and HDPE bottles, respectively, after a storage period of 8 days.

# 3.2. Chemical assessment of milk quality

#### 3.2.1. Lipid oxidation

All milk samples showed a similar pattern of lipid oxidation over the 7 day storage period, despite differences (P < 0.05) exhibited by individual samples (Fig. 1). As expected, the oxidation level generally increased gradually with time in all samples. Between day 3 and day 7 of storage, milk packaged in PET (clear and pigmented) exhibited a higher degree of lipid oxidation (P < 0.05) than the rest of the samples. It is obvious that, in the clear PET, the effect of light was more pronounced than in pigmented materials. This result is supported by spectral transmission curves of packaging materials tested (Fig. 4). The higher degree of lipid oxidation recorded for the pigmented PET than for the pigmented HDPE bottles may be attributed to the much greater thickness of the latter (300-350 µm vs. 550-600 µm), providing a better protection for the product against light. The longer the storage period, the greater was the observed effect of light. What seems interesting is the fact that, within the storage period tested, and for the given packaging materials, oxygen permeability (Table 4) did not seem to significantly influence degree of lipid oxidation in the product. Part of the explanation



Fig. 1. Lipid oxidation of whole pasteurized milk packaged in various containers during storage at 4 °C. White HDPE ( $\blacktriangle$ ), 3 layer pigmented, coextruded HDPE ( $\blacksquare$ ), clear PET ( $\bigcirc$ ), white PET ( $\Box$ ) and coated paperboard carton ( $\blacklozenge$ ).

for this is the relatively large headspace (containing air), which was similar in all packages (25–40 ml), including the "control".

These results are in general agreement with those of Cladman et al. (1998) who, after 7 days of storage, found TBA scores of low fat milk in the range of 0.03–0.15 (absorbance at  $\lambda = 532$  nm) for all packages tested. These authors reported, however, a significantly higher

degree of lipid oxidation in milk packaged in HDPE jugs than in milk packaged in green PET bottles after 7 days of storage. Obviously the green pigmentation PET provided better protection to the product, with respect to light, than partly transparent HDPE. Likewise, Erickson (1997) reported a significantly higher degree of lipid oxidation for samples packaged in HDPE jugs than in samples packaged in paperboard cartons after 8 days of storage. The author attributed such differences to the light transmission properties of the packaging material (Hansen, Turner, & Aurand, 1975).

#### 3.2.2. Lipolysis

Relatively small differences in degree of lipid degradation were observed, between samples stored in the five different packaging materials (Fig. 2). FFA values ranged between 1.5 and 2.1 µ equiv/ml. Generally, a slightly increasing trend was observed for FFA formation as storage time increased. Similar results  $(1.3-2.7 \mu \text{ equiv})$ FFA/ml) were reported by Cladman et al. (1998) for milk packaged in various plastic containers after a storage period of 6 days, and by Celestino, Lyer, and Roginski (1996), who recorded a value of 1.6 µ equiv FFA/ml after 2 days of milk storage. Likewise, Vassila et al. (2002) reported FFA values between 1.9 and 2.7  $\mu$ equiv/ml for milk packaged in various pouch materials. In general, microbial lipases are believed to have an adverse effect on milk fat, only if the psychrotrophic count exceeds 10<sup>7</sup> cells/ml (Law, 1979) which was not the case in the present study. No attempt was made to correlate levels of FFA with psychrotrophic or mesophilic counts, since such a correlation has been reported by Rowe, Johnson, Kilpatrick, Dunstall, and Murphy (1990) to be poor.



Fig. 2. Rate of lipolysis of whole pasteurized milk packaged in various containers during storage at 4 °C. White HDPE ( $\blacktriangle$ ), 3 layer pigmented, coextruded HDPE ( $\blacksquare$ ), clear PET ( $\bigcirc$ ), white PET ( $\Box$ ) and coated paperboard carton ( $\blacklozenge$ ).



Fig. 3. Rate of proteolysis of whole pasteurized milk packaged in various containers during storage at 4 °C. White HDPE ( $\blacktriangle$ ), 3 layer pigmented, coextruded HDPE ( $\blacksquare$ ), clear PET ( $\bigcirc$ ), white PET ( $\Box$ ) and coated paperboard carton ( $\blacklozenge$ ).

#### 3.2.3. Proteolysis

Extent of proteolysis, expressed as released tyrosine residues in whole pasteurized milk packaged in various containers as function of storage time, is shown in Fig. 3. Milk in all packaging materials showed a similar degree of proteolysis in the range 0.010-0.012 mg/ml, which appeared more or less constant with time. Degree of opacity and barrier to O<sub>2</sub> did not affect degree of proteolysis of milk samples. Cladman et al. (1998) reported inconsistent data on proteolysis (tyrosine values ranged from 0.011 to 0.023 mg/ml after a storage period of 6 days) and difficulty in establishing a clear trend with regard to the particular pattern of proteolysis. Vassila et al. (2002) reported similar tyrosine values between 0.007 and 0.013  $\mu$  equiv/ml. Law (1979) reported that tyrosine values in milk exhibited inherent natural variations, with values as high as 0.58 mg/ml, and that milk samples with low initial tyrosine values could undergo considerable proteolysis and yet remain below the threshold value of 0.55 mg/ml (value at which milk producers were penalized for "low quality milk").

## 3.2.4. Vitamin A

Vitamin A contents of whole milk samples packaged in various containers, as a function of storage time, are shown in Table 3. Samples in both HDPE bottles suffered practically the least amount of degradation of vitamin A after 7 days of storage (loss equal to 8.8– 10.5%). Control samples suffered a loss of 14.0%, followed by pigmented PET samples (29.8% loss), and clear PET bottles (50.9% loss). Based on O<sub>2</sub> transmission values (Table 4), headspace volume (from 25 to 40 ml) and headspace oxygen concentration values, from 11 to 21% for the entire 7 day storage period (data not shown), it can be postulated that, within the short life



Fig. 4. Spectral transmission curves of various milk packaging materials. White HDPE (1), 3 layer pigmented, coextruded HDPE (2), clear PET (3), white PET (4) and coated paperboard carton (5).

span of the experiment, oxygen was not a major factor in vitamin A degradation. It is interesting to note that pigmentation with 2% TiO<sub>2</sub> was only partly effective in preventing vitamin A degradation. This finding may be explained by the fact that inorganic filler particles (such as TiO<sub>2</sub>) used to block harmful light wavelengths, may act as strong scattering centres, causing a large proportion of incident light to find its way into the milk at scattered angles (de Man, 1978). Use of the black pigment sandwiched between two white HDPE layers seemed to be unnecessary within the short life span of the product, since monolayer HDPE (550–600  $\mu$ m) provided the same degree of vitamin A protection to the product. This finding is in contrast to that of Vassila et al. (2002), who found better vitamin A protection in milk packaged in pigmented (2% TiO<sub>2</sub>/4% carbon black/ 2% TiO<sub>2</sub>) LDPE pouches than in pigmented (2% TiO<sub>2</sub>) LDPE pouches. Such discrepancies may be interpreted in terms of thickness differences of packaging materials used in the two studies (60–110  $\mu$ m pouches vs. 550–600  $\mu$ m bottles used in the present work). Fanelli et al. (1985) reported losses of vitamin A between 6.3% and 50% after milk irradiation for 24 h with the best protection being achieved through the use of special UV blockers compounded into the HDPE resin used to manufacture plastic bottles. Vassila et al. (2002) similarly reported losses of vitamin A in whole milk of between 15.1% and 73.0% in various flexible pouch materials.

# 3.2.5. Riboflavin

Riboflavin content of whole milk samples in various containers, as a function of storage time, are shown in Table 5. Multilayer and monolayer pigmented HDPE and pigmented PET samples suffered a loss of riboflavin, after 7 day of storage, of 18.4-20.6%, followed by the pigmented PET samples (30.9% loss) and the clear PET samples (47.1% loss). Control samples suffered a loss of 19.8%. It is clear that TiO<sub>2</sub>-pigmented bottles (both HDPE and PET) provided a better protection for riboflavin than the clear bottles (PET). The better protection provided by the pigmented HDPE bottle than the pigmented PET bottle may be associated with the greater thickness of the first than the latter ( $550 \mu m vs. 300-350 \mu m$ ). Fanelli et al. (1985) reported losses of riboflavin between 57% and 70% after 16 h of irradiation

Table 3

Retention of vitamin A in whole pasteurized milk packaged in various containers during storage at 4 °C

| Packaging material                        | Vitamin A (µg/ml)<br>Days of storage at 4 °C |                   |                   |                   |                   |  |
|---|--|-------------------|-------------------|-------------------|-------------------|--|
|   | 0  | 1                 | 3                 | 5                 | 7                 |  |
| 3 layer pigmented, coextruded HDPE bottle | 0.57 <sup>a</sup>                            | 0.56 <sup>a</sup> | 0.55ª             | 0.55ª             | 0.52 <sup>a</sup> |  |
| Monolayer pigmented HDPE bottle           | 0.57 <sup>a</sup>                            | 0.57 <sup>a</sup> | 0.56 <sup>a</sup> | 0.54 <sup>a</sup> | 0.51 <sup>a</sup> |  |
| Clear PET bottle                          | 0.57 <sup>a</sup>                            | 0.51 <sup>a</sup> | 0.43 <sup>b</sup> | 0.36 <sup>b</sup> | 0.28 <sup>b</sup> |  |
| Pigmented PET bottle                      | 0.57 <sup>a</sup>                            | 0.54 <sup>a</sup> | 0.51 <sup>a</sup> | 0.46 <sup>c</sup> | 0.40 <sup>c</sup> |  |
| Coated paperboard carton                  | 0.57 <sup>a</sup>                            | 0.57 <sup>a</sup> | 0.57 <sup>a</sup> | 0.54 <sup>a</sup> | 0.49 <sup>a</sup> |  |

<sup>a,b,c</sup> Values within a column followed by different letters are significantly different (P < 0.05). Values reported are the means of six replicates (n = 6).

#### Table 4

Oxygen transmission rate of packaging materials

| Packaging material                        | O2 transmission rate (ml/package·day·atm) <sup>a</sup> |
|---|--|
| 3 layer pigmented, coextruded HDPE bottle | 1.9  |
| Monolayer pigmented HDPE bottle           | 2.0  |
| Clear PET bottle                          | 0.8  |
| Pigmented PET bottle                      | 0.7  |
| Coated paperboard carton                  | 34.2   |

Values reported are the means of six replicates (n = 6).

<sup>a</sup> T = 22 °C, RH = 60%.

| Table : |
|---------|
|---------|

Retention of riboflavin in whole pasteurized milk packaged in various containers during storage at 4 °C

| Packaging material                        | Riboflavin (μg/ml)<br>Days of storage at 4 °C |                   |                   |                   |                   |  |
|---|---|-------------------|-------------------|-------------------|-------------------|--|
|   | 0   | 1                 | 3                 | 5                 | 7                 |  |
| 3 layer pigmented, coextruded HDPE bottle | 1.36 <sup>a</sup>                             | 1.30 <sup>a</sup> | 1.25 <sup>a</sup> | 1.19 <sup>a</sup> | 1.11 <sup>a</sup> |  |
| Monolayer pigmented HDPE bottle           | 1.36 <sup>a</sup>                             | 1.30 <sup>a</sup> | 1.23 <sup>a</sup> | 1.15 <sup>a</sup> | 1.08 <sup>a</sup> |  |
| Clear PET bottle                          | 1.36 <sup>a</sup>                             | 1.14 <sup>a</sup> | 1.03 <sup>b</sup> | 0.92 <sup>b</sup> | 0.72 <sup>b</sup> |  |
| Pigmented PET bottle                      | 1.36 <sup>a</sup>                             | 1.20 <sup>a</sup> | 1.15 <sup>a</sup> | 1.04 <sup>c</sup> | 0.94 <sup>c</sup> |  |
| Coated paperboard carton                  | 1.36 <sup>a</sup>                             | 1.29 <sup>a</sup> | 1.20 <sup>a</sup> | 1.15 <sup>a</sup> | 1.09 <sup>a</sup> |  |

<sup>a,b,c</sup> Values within a column followed by different letters are significantly different (P < 0.05).

Values reported are the means of six replicates (n = 6).

using a variety of pigments and UV absorbers in their HDPE containers. de Man (1978) reported riboflavin losses, after exposure of milk containers to fluorescent light for 48 h, equal to 16.6% for paperboard cartons, 28.4% for clear PE pouches, 18.8% for HDPE jugs and 15.3% for jugs pigmented with 2% TiO<sub>2</sub>. Hoskin and Dimick (1979) reported riboflavin losses after exposure of milk containers to fluorescent light for 72 h equal to 13% for clear PC bottles, 10% for HDPE bottles, 10% for paperboard cartons and 6% for PC tinted bottles. No significant loss of riboflavin was observed in milk held in the dark. Finally, Vassila et al. (2002) reported losses in vitamin B<sub>2</sub> of between 18.8% and 45.3% for whole milk after storage for 7 days in various plastic pouch materials.

A final important point to be made is that the two main mechanisms of milk quality deterioration are chemical oxidation through  $O_2$  permeation and lightinduced oxidation/vitamin degradation. The chemical nature and thickness (300–600 µm) of the packaging material may effectively control the first mechanism (given a reasonably small headspace), while TiO<sub>2</sub> and/or carbon black pigmentation may partly control only the latter.

#### 3.2.6. Sensory evaluation

Flavour evaluation results are shown in Table 6. In general, oxidation off-flavours increased more rapidly in

milk packaged in light-exposed containers than in lightprotected containers. After 5 days of storage, the milk sample in both the pigmented HDPE bottles received a score of 4.2, followed by the sample in the paperboard carton (score 3.6) and that in the pigmented PET bottles (score 3.5). Samples in the clear PET bottles were unacceptable (score 2). Flavour defects were described as "slightly stale" for samples packaged in HDPE and paperboard cartons and "plastic"/"burnt taste" for samples packaged in PET bottles. After 7 days of storage, milk was unacceptable in all containers.

The present sensory results are in agreement with those of van Aardt et al. (2001) who reported better milk quality retention in amber PET and UV compounded PET than in clear PET bottles; and those of Cladman et al. (1998) who reported better milk protection against light oxidation when packaged in green PET than in clear PET bottles. What should be stressed at this point is that, even though the total microbial count was in all cases below or equal to the "so called" upper limit for acceptability,  $10^6-10^7$  cfu/ml, the organoleptic character of the product had clearly deteriorated, resulting in an unacceptance product. This has previously been noted by Labuza (1982) and Maxcy and Wallen (1983), showing that the most dependable method for judging the quality of fresh milk is sensory evaluation, since the type of spoilage organisms rather than total organism population is the decisive factor in milk spoilage.

Table 6

Flavour evaluation of whole pasteurized milk packaged in various containers during storage at 4 °C

| Packaging material                        | Score/comments<br>Days of storage at 4 °C |                |                                 |                                  |                              |  |
|---|---|----------------|---------------------------------|----------------------------------|------------------------------|--|
|   | 0   | 1              | 3                               | 5                                | 7                            |  |
| 3 layer pigmented, coextruded HDPE bottle | 5 <sup>a</sup>                            | 5 <sup>a</sup> | 5 <sup>a</sup>                  | 4.2 <sup>a</sup>                 | 1.1 <sup>a</sup> /sour       |  |
| Monolayer pigmented HDPE bottle           | 5 <sup>a</sup>                            | 5 <sup>a</sup> | 5 <sup>a</sup>                  | 4.2 <sup>a</sup>                 | 1.2 <sup>a</sup> /sour       |  |
| Clear PET bottle                          | 5 <sup>a</sup>                            | 5 <sup>a</sup> | 3.2 <sup>b</sup> /plastic taste | 2.0 <sup>b</sup> /plastic taste, | 0 <sup>b</sup> /unacceptable |  |
| Pigmented PET bottle                      | 5 <sup>a</sup>                            | 5 <sup>a</sup> | 4.2 <sup>c</sup>                | 3.5°/slightly stale              | 1ª/unacceptable              |  |
| Coated paperboard carton                  | 5 <sup>a</sup>                            | 5 <sup>a</sup> | 5 <sup>a</sup>                  | 3.6°/slightly stale              | 2 <sup>c</sup> /stale, sour  |  |

<sup>a,b,c</sup> Values within a column followed by different letters are significantly different (P < 0.05).

Numerical scale of scoring: very good = 5, good = 4, fair = 3, poor = 2, very poor = 1, unfit for consumption = 0.

## 4. Conclusions

All packaging materials tested provide sufficient protection with regard to microbial growth, as well as degree of lipid oxidation, lipolysis and proteolysis, in pasteurized whole milk stored under fluoroscent light and refrigeration for a period of 7 days. Bottles made of pigmented (TiO<sub>2</sub>) HDPE at a thickness of 550-600 µm effectively protected milk against vitamin A degradation. Pigmented (TiO<sub>2</sub>) PET bottles at a thickness of 300-350 µm provided only partial protection, while clear PET provided the least amount of protection of milk against vitamin A degradation. A similar pattern was recorded for riboflavin. Based on organoleptic evaluation, the shelf life of whole pasteurized milk in Greece is 5 days. The best overall protection of the product was provided by the multilayer and monolayer HDPE bottle. Evidently, either multilayer (TiO<sub>2</sub>/black pigment/TiO<sub>2</sub>) pigmented or monolayer (TiO<sub>2</sub>) pigmented HDPE, 550–600 µm in thickness, may be used as attractive and convenient alternatives to the coated paperboard carton for fresh milk packaging.

#### Acknowledgements

The authors thank ARGO Ltd. for providing the HDPE and PET containers and DODONI Ltd. for providing the milk samples.

#### References

- Allen, J. C., & Joseph, G. (1985). Deterioration of pasteurized milk on storage. *Journal of Dairy Research*, 52, 469–487.
- Bakish, R. & Hatfield, E. (1997). Coextrusions for flexible packaging. In A. L. Brody & K. S. Marsch (Eds.), *Wiley Encyclopedia of Packaging Technology*, (2nd ed., pp. 237–240 and 629–638). New York: John Wiley and Sons.
- Barnard, S. E. (1972). Importance of shelf life for consumers of milk. *Journal of Dairy Science*, 55, 134–136.
- Celestino, E. L., Lyer, M., & Roginski, H. (1996). The effects of refrigerated storage on the quality of raw milk. *Australian Journal* of Dairy Technology, 51, 59–63.
- Cladman, W., Scheffer, S., Goodrich, N., & Griffiths, M. W. (1998). Shelf life of milk packaged in plastic containers with and without treatment to reduce light transmission. *International Dairy Journal*, 8, 629–636.
- de Man, J. M. (1978). Possibilities of prevention of light-induced quality loss of milk. *Journal of the Canadian Institute of Food Science and Technology*, 11, 152–154.
- Deeth, H. C., Fitzgerald, C. H., & Wood, A. F. (1975). A convenient method for determining the extend of lipolysis in milk. *Australian Journal of Dairy Technology*, 30, 109–111.
- Defosse, M. (2000). Novel milk bottle designs keep blow molders on top. *Modern Plastic International* (October), 114–116.
- Erickson, M. (1997). Chemical and microbial stability of fluid milk in response to packaging and dispensing. *International Journal of Dairy Technology*, 50, 107–111.

- Fanelli, A. J., Burlew, J. V., & Gabriel, M. K. (1985). Protection of milk packaged in high density polyethylene against photodegradation by fluorescent light. *Journal of Food Protection*, 48, 112– 117.
- Hansen, A. P., Turner, L. G., & Aurand, L. W. (1975). Fluorescent light activated flavor in milk. *Journal of Milk Food Technology*, 38, 388–391.
- Hoskin, J. C., & Dimick, P. S. (1979). Evaluation of fluorescent light on flavor and riboflavin content of milk held in gallon returnable containers. *Journal of Food Protection*, 42, 105–109.
- Hull, M. E. (1947). Colorimetric determination of the partial hydrolysis of the proteins in milk. *Journal of Dairy Science*, 30, 881–884.
- IDF (International Dairy Federation). (1986). Bulletin 200. Pasteurization.
- IDF, (1987). 99A. Sensory evaluation of dairy products.
- IDF, (1991a). Enumeration of microorganisms; colony count technique at 30 °C, Standard 100 B.
- IDF, (1991b). Estimation of numbers of psychrotrophic microorganisms. Rapid colony count technique, 25 h at 21 °C, Standard 132 A.
- King, R. L. (1962). Oxidation of milk fat globule membrane material. Thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. *Journal of Dairy Science*, 45, 1165–1171.
- Labuza, T. P. (1982). *Open shelf-life dating of foods*. Westport CT: Food and Nutrition Press.
- Law, B. A. (1979). Reviews of the progress of Dairy Science: Enzymes of psychrotrophic bacteria and their effects on milk and milk products. *Journal of Dairy Research*, 46, 573–588.
- Marsili, R. T. (1999). Comparison of SPME and dynamic headspace method for the GC-MS analysis of light-induced lipid oxidation products in milk. *Journal Chromatography Science*, 37, 17–23.
- Maxcy, J. R., & Wallen, S. E. (1983). Heterogeneity of samples as a problem in shelf life prediction. *Journal of Food Protection*, 46, 543–544.
- Ravanis, S., & Lewis, M. J. (1995). Observations on the effect of raw milk quality on keeping the quality of pasteurized milk. *Letters in Applied Microbiology*, 20, 164–167.
- Rowe, M. T., Johnson, D. E., Kilpatrick, D. J., Dunstall, G., & Murphy, R. J. (1990). Growth and extracellular enzyme production by psychrotrophic bacteria in raw milk stored at low temperature. *Milchwissenschaft*, 45, 495–498.
- Rysstad, G., Ebbesey, A., & Eggestad, J. (1998). Sensory and chemical quality of UHT milk stored in paperboard cartons with different oxygen and light barriers. *Food Additives and Contaminants*, 15(1), 112–122.
- Sattar, A., de Man, J. M., & Alexander, J. C. (1977). Wavelength effect on light-induced decomposition of vitamin A and beta-carotene in solutions and milk fat. *Journal of the Canadian Institute of Food Science and Technology*, 10, 56–60.
- Schröder, M. J. A. (1982). Effect of oxygen on keeping the quality of milk. *Journal of Dairy Research*, 49, 407–424.
- Schröder, M. J. A., Scott, K. J., Bland, K. J., & Bishop, D. R. (1985). Flavor and vitamin stability in pasteurized milk in polyethylenecoated cartons and in polyethylene bottles. *Journal of the Society of Dairy Technology*, 38, 48–52.
- Skibsted, L. H. (2000). Light induces changes in dairy products. Bulletin of the International Dairy Federation, 345, 4–9.
- Thomas, E. L. (1981). Trends in Milk Flavors. Journal of Dairy Science, 64, 1023–1027.
- Toyosaki, T., Yamamoto, A., & Mineshita, T. (1988). Kinetics of photolysis of milk riboflavin. *Milchwissenschaft*, 43, 143–146.
- Valero, E., Villamiel, M., Sanz, J., & Martinez-Castro, I. (2000). Chemical and sensorial changes in milk quality on keeping the quality of pasteurized milk. *Letters in Applied Microbiology*, 20, 164–167.
- Vassila, E., Badeka, A., Kondyli, E., Savvaidis, I., & Kontominas, M. G. (2002). Chemical and microbiological changes in fluid milk as

affected by packaging conditions. International Dairy Journal, 12, 715–722.

- van Aardt, M., Duncan, J. S. E., Marcy, E., Long, T. E., & Hackey, C. R. (2001). Effectiveness of poly(ethylene terephthalate) and highdensity polyethylene in protection of milk flavor. *Journal of Dairy Science*, *84*, 1341–1347.
- White, C. H. (1985). Consumer reaction to colored plastic milk jugs. *Journal of Dairy Science*, 68, 261–264.
- Zahar, M., & Smith, D. E. (1990). Vitamin A quantification in fluid dairy products: Rapid method for Vitamin A extraction for high performance liquid chromatography. *Journal of Dairy Science*, 73, 3402–3407.