Storage of pasteurized milk in clear PET bottles combined with light exposure on a retail display case: a possible strategy to define the shelf life and support a recyclable packaging

Sara LIMBO*, Luisa PELLEGRINO, Paolo D'INCECCO, Serena GOBBI, Veronica ROSI, Daniela FRACASSETTI

Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Milan, Italy

Corresponding author at Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Via G. Celoria 2, 20133 Milan, Italy. E-mail address: sara.limbo@unimi.it. Phone: +39 0250316659

Abstract

The stability of whole pasteurized milk packaged in clear PET bottles was studied throughout 13-days storage in the dark, but including, at specific times, light exposure of 6, 12 or 18 hours to simulate conditions potentially occurring in refrigerated display counters. The aim was to investigate the effects of light exposure when overlapping the unavoidable endogenous modifications in pasteurized milk during storage. Dissolved oxygen, riboflavin and other flavins, proteolysis products, volatile compounds, and sensory characteristics were evaluated. Besides the expected progress of proteolysis occurring during storage, light negatively affected milk flavour especially after longer exposure times. The development of “mushroom” flavor related to the increase of volatile 2,3 octanedione was the most characterizing modification. Gathered data were considered in view of providing the background knowledge for the control of light exposure conditions on a retail display, thus supporting the shelf life extension of pasteurized milk in a fully recyclable packaging.

Keywords: pasteurized milk; clear PET bottle; retail display; shelf life extension; riboflavin; proteose peptones; volatile compounds; sensory analysis.

1. Introduction

Milk is a highly consumed food product due to its content of valuable nutrients and relatively low price. As compared with the longer shelf stable UHT milk, consumers looking for fresh tasting foods usually prefer pasteurized milk. However, the most seriously limiting factor for widespread consumption of pasteurized milk is that it must be stored under refrigerated conditions. The shelf life of pasteurized milk largely depends on the microbiological quality of raw milk. Milk acidification, due to lactic acid fermentation, as well as proteolysis and lipolysis due to heat resistant microbial enzymes are well-established phenomena contributing to milk decay during storage (Murphy, Martin, Barbano, & Wiedmann, 2016; Ziyaina, Govindan, Rasco, Coffey, & Sablani, 2018). Other enzymes native of milk or bound to somatic cells take part in the degradation of milk components. In particular, the most important protease in milk, i.e. plasmin, is not inactivated by pasteurization and thus its activity continues in the finished product during storage with a negative impact on sensory properties of milk (Cattaneo, Stuknyté, Pellegrino, & De Noni, 2014). Besides the selection of high quality raw milk in terms of bacterial and somatic cells counts, as provided by regulatory bodies worldwide (EC, 2004; FDA, 2015), technological interventions have been proposed to remove these degradative agents from milk before pasteurization. Both centrifugation and microfiltration (D'Incecco, Rosi, Cabassi, Hogenboom, & Pellegrino, 2018), proved to allow effective cleaning of raw milk thus extending the shelf life of the derived consumption milk.

Producing commercial pasteurized milk with a longer shelf life has measurable economic advantages for manufacturers and allows reducing food waste due to premature spoilage. Extending the shelf life, however, increases the time the bottled milk may stay on the display counter in the shops. Although the low temperature slows down both bacterial growth and enzymatic activities, when milk is exposed to light at wavelengths in the UV-visible range, such as those of the lamps commonly used by the mass retail channels can activate complex photo-

53 degradative reactions responsible for several negative changes (Wold, Skaret, & Delsgaard, 2015).
54 Photo-oxidation takes place by either photolytic autoxidation (i.e. the UV-induced production of
55 free radicals, primarily from lipids) or photosensitized oxidation (that occurs in the presence of
56 photosensitizers). The latter route mainly characterizes the photo-oxidation in bottled milk and it
57 involves oxygen, that can reach 6-7 mg/L, and photo-sensitive molecules, like riboflavin (vitamin B2,
58 1-2 mg/L in cow's milk) or other minor constituents, such as protoporphyrin IX, hematoporphyrin,
59 and tetrapyrroles (Airado-Rodríguez, Intawiwat, Skaret, & Wold, 2011; Fracassetti, Limbo,
60 D'Incecco, Tirelli, & Pellegrino, 2018). The detrimental effects of light and oxygen in foods have been
61 mainly associated to sensory changes and the derived defect is known as "sunlight flavor" (Airado-
62 Rodríguez et al., 2011). The presence of sulphur compounds originating from degradation of
63 methionine, has been reported to be responsible for this fault in high-temperature treated milk
64 (Beauchamp, Zardin, Silcock, & Bremer, 2014) but also in wine (Fracassetti, Gabrielli, Encinas,
65 Manara, Pellegrino, & Tirelli, 2017) and beer (Landaud, Helinck, & Bonnarme, 2008). Carbonyl
66 compounds, such as aldehydes and ketones derived from fatty acids, also affect the volatile profile
67 of milk, depending on its fat content (Beauchamp et al., 2014).

68 The possibility to prevent light-induced defects has been primarily entrusted to packaging materials.
69 The combination of plastic resins with color pigments and UV absorbers offers a good barrier to light
70 (Mestdagh, De Meulenaer, De Clippeleer, Devlieghere, & Huyghebaert, 2005). Recently, Wang et al.
71 (2018) demonstrated that the combination of a light-protective additive (TiO_2) and oxygen barrier
72 material successfully reduces the formation of oxidation products in milk. Pasteurized milk is usually
73 packaged either in clear or colored bottles, made by polyethylene terephthalate (PET) or high-
74 density polyethylene (HDPE), or in paperboard cartons. Intawiwat et al. (2013) demonstrated that
75 to preserve milk quality, the light transmission for wavelength below 450 nm and above 650 nm
76 should be minimized, thus a modulated filter with adequate characteristics must be used in the

77 formulation of the polymer. However, the clear PET bottle is usually preferred by the consumers
78 since they like seeing the milk inside and, concomitantly, by producers since it represents an
79 interesting packaging solution to support the concepts of “environmental-friendly” and “circular
80 economy”. In fact, the unmatched opportunities offered by the closed-loop recycling of PET bottles
81 into new food-grade PET containers extend the value of this synthetic polymer, reducing the
82 material and energy burdens that affect the plastic industry (Hahladakis & Iacovidou, 2018). Other
83 targeted strategies limiting the photo-oxidation of bottled milk would be the reduction or
84 modulation of the light emitting spectra of lamps (Webster, Duncan, Marcy, & O’Keefe, 2009) and
85 the optimization of the turnover time of bottled milk on the lighted shelves (Chang & Dando, 2018).
86 The effects of discrete exposition of milk to fluorescent or LED lamps have been investigated during
87 the last years (Brothersen, McMahon, Legako, & Martini, 2016; Wang, Duncan, Whalley, & O’Keefe,
88 2020) but, to date, little information is available on the chemical and sensory changes that occur in
89 packaged milk repeated illumination periods (Wang et al., 2018) during its entire shelf life, thus as
90 its storage progresses.

91 The aim of this research was to investigate the effects of the light exposure when overlapping the
92 unavoidable endogenous modifications arising in pasteurized milk during the shelf storage. Thus,
93 the progress of selected quality indicators was monitored in pasteurized milk packaged in clear PET
94 bottles for a longer period (13-days) than standard, including -at specific times- lighting up to a total
95 of 18 hours. Data gathered with this study were considered in view of providing the background
96 knowledge for a possible shelf life extension of pasteurized milk in clear PET bottles during real
97 conditions of market storage.

98

99 **2. Materials and Methods**

100 **2.1. Milk samples**

101 Commercial full-fat (35 g/L) pasteurized cow's milk was produced at an industrial plant from raw
102 bulk milk collected from local farms the day before that of processing and stored at 4°C overnight.
103 The manufacturer provided data from raw milk testing for total bacteria count ($1.2x10^4$ CFU/mL)
104 and somatic cell count ($2.17x10^5$ cells/mL). Pasteurization was carried out using a commercial plant
105 and pasteurized milk was in-line filled into 1 L bottles of clear PET with HDPE cap. The shelf life
106 assigned by manufacturer was 7 days at 4-6°C. Sets of 12 bottles were shrink-wrapped with a black
107 thermo-retractable PVC film to avoid light exposure during transportation and storage. A total of
108 156 bottles were brought to the laboratory under refrigerated (5±1°C) conditions on the day of
109 production.

110 **2.2. Experimental design**

111 The packaging treatment was the same for all the tested samples (PET bottle). The milk bottles were
112 basically kept protected from light in a cold (4±1°C) storage room up to 13 days. The packaging
113 treatment was the same for all the tested samples (PET bottle). The milk bottles were basically
114 kept protected from light in a cold (4±1°C) storage room up to 13 days. At 5 intervals (n=5)
115 during storage in dark conditions (2, 3, 7, 8, 13 days), some bottles were moved to the display
116 and stored for different lighting intervals equal to 6, 12 and 18 hours (coded as 6h, 12h and
117 18h). Therefore, at each storage time, 4 treatments were considered (n=4): milk never exposed
118 to light and milk exposed to light for 6, 12 and 18h, respectively. Three replications were carried
119 out for this study. An open-front refrigerated (5±1°C) and vertical retail case with four shelves
120 was used (Arrigoni, Italy) and the display was placed in a dark and conditioned room at 25°C,
121 to minimize any interference from the external environment. When in the display, milk bottles
122 were periodically turned in order to achieve the same light exposure. The applied exposure
123 conditions were intended to simulate the conditions of daily display of bottled milk on the shelf of
124 a big retail shop. The actual age of the milk at sampling included both the time spent in the storage

125 room and the lighting period on the display. For each sampling time, three bottles were used for the
126 determination of dissolved oxygen and for the sensory analysis, and three bottles were analysed for
127 the content of riboflavin and other flavones, volatile compounds, peptones and small peptides, and
128 free amino acids (Figure S1). Total viable bacterial count of milk was determined in bottles at 13-
129 day storage by agar plate count (International Dairy Federation standard 100B, 1991), and values
130 were always lower than 10^4 CFU/mL, i.e. the threshold value the manufacturer accepts at the expire
131 date.

132 **2.3. Light exposure conditions and light spectrum of the lamp in the retail shelf**

133 Fluorescent tubular lamps TL-D super 58W/840 (Philips, Italy) characterized by a color temperature
134 of 4000 K were used in the experiment. The lamps were placed horizontally in a vertical display
135 cabinet (Costan, Italy), one on each of the three shelves, about 15 cm above the bottles. The spectral
136 irradiance (W m^{-2}) of the lamp was measured by a spectrophotometer (Konica Minolta, mod. CL-
137 500 A) in 10 different positions of the different shelves.

138 **2.4. Dissolved oxygen**

139 The amount (mg/L) of dissolved oxygen in milk was measured in all of the bottles of this study. An
140 oximeter equipped with a temperature sensor (Oxi 340i/SET, WTW, Germany) was used and
141 measurement was always done at the same depth within the bottle.

142 **2.5. Determination of proteolysis indicators**

143 The contents of proteose peptones and small peptides were determined by HPLC-UV in accordance
144 to Pellegrino et al. (2015). Briefly, milk (20 mL) was adjusted to pH 4.6 with 2N hydrochloric acid and
145 then centrifuged at 5000*g* for 20 minutes. The supernatant was filtered through a 0.22 µm PVDF
146 filter (Millipore, Ballerica, MA, USA) and analysed using a Waters Alliance 2695 chromatograph
147 (Waters, Milford, MA, USA) equipped with a 2996 diode array detector (Waters, USA) and a PLRP-S

chromatographic column (250 x 4.6 mm, 300 Å pore size, 5 µm particle size) (Varian Medical System, Milan, Italy) set at 40°C. Chromatographic data were processed at 205 nm using Empower2 software (Waters).

Free amino acids were analysed on the filtrate prepared as described above using the ion-exchange chromatography (IEC) and post-column derivatization with nynhydrin. The analytical conditions were as described by Hogenboom et al. (2017). A Biochrom 30plus amino acid analyser (Biochrom, Cambridge, UK) was used and the elution conditions were those recommended by the manufacturer. Peaks were identified by comparison with a standard and quantitation was performed using four-point calibration curves.

2.6. Determination of riboflavin and flavones

Riboflavin, flavin mononucleotide, flavin adenine dinucleotide, lumiflavin and lumichrome were determined by HPLC and fluorescence detection (excitation: 420 nm; emission: 530 nm) as described by Fracassetti et al. (2018). Briefly, milk (2 mL) was skimmed by centrifugation (18,000 *g*/30 min/5 °C) (benchtop centrifuge, Hettich, Tuttlingen, Germany) and ultrafiltered with a disposable 10 kDa cut-off membrane Microcon (Millipore). Samples were protected from light during preparation. The HPLC equipment was a Waters Alliance 2695 (Milford) equipped with an ODS Hypersil chromatographic column (100x3 mm, 3 µm particle size) (CPS Analytica, Milan, Italy) set at 40 °C. Elution and quantitation conditions corresponded to those reported by Fracassetti et al. (2018). Chromatographic data were processed using Empower2 software (Waters).

2.7. Determination of volatile compounds

The volatile compounds were sampled by a headspace solid phase micro extraction (SPME) technique followed by gas chromatography−mass spectrometry (GC-MS). Ten mL of milk sample were added with 3 g of sodium chloride and d5-chlorobenzene dissolved in ethanol (25 µg/L; Sigma-

171 Aldrich, Milan, Italy) as internal standard in hermetically closed glass-vial provided with a pierceable
172 septum (HTA, Brescia, Italy). The fibre used was a carboxen-polydimethylsiloxane-divinylbenzene
173 (CAR-PDMS-DVB; 50/30 µm x 1 cm) (Supelco, Bellefonte, PA, USA). The SPME was automatically
174 carried out by means of an autosampler (HTA) set at the following conditions: incubation for 10 min
175 at 40°C; agitation for 5 min; extraction for 45 min; desorption for 20 min. The GC-MS equipment
176 was a Perkin Elmer Autosystem XL Gas Chromatograph coupled with a Turbomass Mass
177 Spectrometer (Perkin Elmer, Italy). The injector was set at 250°C and the injection mode was splitless
178 for 0.75 min. The gas-chromatographic separation was carried out with DB-5MS UI (30m x 0.250
179 mm x 0.25 µm; Restek, Bellefonte, PA, USA) using helium as carrier at flow rate of 1.2 mL/min. The
180 oven temperature was initially set at 40°C and held for 4 min, ramped at 5°C/min up to 120°C and
181 held for 5 min, finally ramped at 20°C/min up to 240°C and held for 2 min. The transfer line
182 temperature was set at 200°C and the source temperature at 250°C. The mass spectrometer
183 operated in electron ionization mode at 70 eV using full scan mode. The MS detector registered the
184 *m/z* in the range from 35 Da up to 350 Da. The ions used for identification were chosen according
185 to the NIST MS Search 2.0 library and validated by external standard comparisons of ion
186 fragmentation patterns. Relative abundances of each volatile compound were determined through
187 the ratio of the target compound areas and the internal standard area (A/Ais), as reported by
188 Brothersen et al. (2016). Triplicate analyses were carried out for each sample.

189 **2.8. Sensory analysis**

190 A panel of 7 expert judges (5 females and 2 males, aged between 24 and 54 years) was enrolled for
191 the sensory analysis of milk samples. The attributes related to the qualitative description of the
192 light-exposed milk were identified by the consensus method (ISO 11035, 1994). The selected
193 descriptors were “typical milk flavor”, “mushroom”, “garlic, cabbage”, “rancid, soapy”. The panel
194 was calibrated by using samples of milk exposed to light for 6, 12, 18 and 24 hours. For the

quantitative analysis, judges were asked to smell and taste the milk samples and to assign a score on a scale from 1 (not perceived) to 5 (extremely perceived). Each judge had a different randomized order of samples to test.

2.9. Statistical analysis

All the chemical analyses were conducted in triplicate for all the tested conditions. Statistical analysis was carried out by means of SPSS Win 12.0 software (SPSS Inc., Chicago, IL). One-way ANOVA was performed considering separately the increasing duration of either light-exposure or the storage time of milk. In this way, either light-dependent changes or endogenous modification of milk can be highlighted. Differences among light treatments (n=4) and among storage times (n=5) were tested by the Fisher test (Least Significant Difference, LSD) and the significance level was set at $p < 0.05$.

For the sensory data, the Partial Least Square (PLS) analysis was also carried out as regard of the time of both light exposure and storage of milk. The Variable Importance in Projection (VIP) scores were estimated in order to understand the importance of the selected descriptors on the sensory changes of milk due to the combined effect of light exposure and storage. In order to achieve an overall evaluation of milk changes as function of light exposure and storage, the Principal Component Analysis (PCA) was carried out on the chemical, sensory and microbiological data. Before PCA, data were pre-processed using the auto-scale mode and transformed using the normalized method. The Unscrambler v.9.7 software (Camo Software AS, 2007, Oslo, Norway) was used.

The PCA was performed and a $S \in R^{NxK}$ matrix was generated, where S is the score, N is the number of sampling points (milk stored in the dark and at increasing time under light) and K the number of variables used in the study. The scores of higher-order PCs have been used to investigate the

relationship with light exposure and storage time, while the loadings to reveal the key attributes responsible for the product degradation, under the different conditions of storage. At each sampling time, the relationship between the multivariate quality parameter Q (represented by each PC1 value obtained in the PCA) and the time of exposure *t* was derived as described by the following equation (1):

$$\frac{dQ}{dt} = k_m Q^n \quad (1)$$

where k_m is the multivariate rate constant and *n* is the reaction order.

3. Results and Discussion

3.1 Characterization of the lighting system

The emission spectrum of the fluorescent lamp used in this study was first evaluated. As shown in Figure 1a, the spectral irradiance profile displayed main emission peaks at 408, 437, 549 and 582 nm that were maintained when the PET film of our milk bottles was inserted between the light source and the spectrophotometer. This confirmed that the clear PET does not filter any specific wavelengths in the visible range (Wang et al., 2018). The bottles were periodically moved along the shelf to avoid that they receive a different flux of energy depending on the position (Figure 1b).

3.2 Oxygen consumption

The content of dissolved oxygen in milk slightly decreased, from 6.92±0.04 mg/L to 6.03±0.16 mg/L, during dark storage ($p>0.05$). The oxygen consumption in absence of light can be ascribed to the physiological endogenous reactions that occur in a pasteurized milk, due to the presence of microorganisms and enzymes (Schröder, 1982). At the same time, permeability characteristics of the packaging materials, especially of HDPE cap, do not completely prevent the entrance of oxygen

into the bottle, maintaining quite constant the gas concentration in the milk. Light exposure had an important influence on oxygen consumption in milk samples, as recently described (Wang et al., 2018). Oxygen concentration decreased exponentially with increasing time of light exposure, being values halved after 18 hours. The oxygen consumption followed a first order kinetic independently of the milk age, and the rate of consumption was significantly higher at 13d of storage (p=0.0387) in comparison to 3d (Figure S2). Interestingly, as storage increased, the curve of oxygen consumption rate moved towards lower oxygen concentrations, although the content of dissolved oxygen after 6 and 12 hours of light exposure was not significantly different among the samples with 3 and 8 days of storage (p>0.05).

3.3 Proteolysis progress

Proteose peptones (PP) directly derive from the proteolytic activity of plasmin on β-casein. Since plasmin is stable to milk pasteurization, the progress of this proteolytic activity was associated to the quality decay of pasteurized milk during storage (Murphy et al., 2016). For this reason, a PP concentration not higher than 900 mg/L was proposed for characterizing pasteurized milk that maintains acceptable quality during the shelf life (De Noni, Pellegrino, Cattaneo, & Resmini, 2007). In long stored pasteurized milk, PP are further degraded into small peptides (SP) by bacterial proteases (Cattaneo et al., 2014). Based on this knowledge, both PP and SP were taken in this study as descriptors of proteolysis progress, thus of milk storage itself, owing to the fact that their content was not influenced by light exposure regardless the age of milk (p > 0.79 for PP and p > 0.99 for SP) (Figure S3). As expected, the content of PP increased linearly (r=0.99) during storage (data not shown), as a consequence of plasmin activity, and approached the threshold value of 900 mg/L at 13 days of dark storage, that is 6 days beyond the shelf life indicated by the manufacturer. In contrast, the content of SP did not increase significantly due to the good microbiological quality of the raw milk used by the manufacturer.

The lack of a relevant microbial protease activity also explains why the content of free amino acids did not change significantly during milk storage. In fact, the total levels of free amino acids were 81.7±1.0 and 81.2±1.1 mg/L at 13 days of storage in the dark and with 18-hour lighting, respectively. These data were not significantly different ($p > 0.05$) from the initial (day 1) level of 82.1±1.0 mg/L. In particular, no changes were observed in free methionine content that was always lower than 0.1 mg/L (not shown), in agreement with literature data (Pellegrino et al., 2015). These low levels suggest that, although this amino acid is sensitive to light oxidation (Min & Boff, 2002), its contribution to the development of sulfur-containing compounds responsible of off-flavors can be considered negligible. No free cysteine was detected.

3.4 Riboflavin and other flavones

The content of both riboflavin (RF) and flavin mononucleotide (FMN) remained unchanged in the control samples (dark storage) up to 13 days (Table 1), confirming the stability of these compounds in absence of a light source (Sheraz, Kazi, Ahmed, Anwar, & Ahmad, 2014). Contrarily, both flavins slowly degraded as the cumulative time of light exposure increased, reaching levels significantly different from the control after 18 hours of lighting. No flavin adenine dinucleotide was detected, while a higher concentration of RF than those found in Fracassetti et al. (2018) was determined. Both RF and FMN are involved in photo-oxidation reactions (Choe, Huang, & Min, 2005). The light exposure can cause the cleavage of the ribityl group of excited triplet RF leading to the production of several compounds, i.e. lumichrome (LC) and, to a lesser extent, lumiflavin (LF) (Sheraz et al., 2014). These two compounds are also photosensitizers and seem to have aptitude to form singlet oxygen comparable to those of RF, thus may equally contribute to decay of sensory quality of milk (Huang, Kim, & Min, 2006). Both LF and LC contents increased upon light exposure, with a significant difference from the control observed even after 6 hours lighting. Little increase of LF was found in comparison to LC indicating that LF is a minor compound at pH close to neutrality (Huang et al.,

288 2006). The increased age of milk did not affect the degradation rate of both RF and FMN as well as
289 the formation of LF and LC.
290
291 **3.5 Volatile compounds**

292 Volatile compounds (VOCs) analysis evidenced the prevailing presence of distinct short-chain (C5-
293 C9) saturated aldehydes and ketones. The relative abundance increased from the second day
294 onwards (Figure 2), in particular for hexanal, which is described having green, fatty, leafy, vegetative
295 and fruity flavor, and heptanal, having strong fatty, harsh, pungent flavor (Brothersen et al., 2016).
296 These compounds are often associated to off-flavor derived from lipid oxidation (Johnson, Duncan,
297 Bianchi, Chang, Eigel, & O'Keefe, 2015), specifically, their origin can be found in the degradation
298 reactions of hydroperoxides, which in turn may derive from radical oxidation of unsaturated fatty
299 acids (Lee & Min, 2009). Formation of octanal (fatty, citrus and honey flavor) and nonanal (fatty,
300 orange and rose flavor) was also detected with similar trends but at lower relative abundances. The
301 increase of all these compounds in dark stored samples was observed starting from the eighth day
302 of storage, but it did not affect the off-flavor perception, as it was confirmed by sensory analysis
303 data. The development of the observed volatile compounds became more important in light
304 exposed samples, starting from the eighth day of shelf life, reaching the highest relative abundance
305 at 13 days of storage in the dark but showing a significant difference after a total of 18 hours lighting.
306 It is noteworthy that the presence of 2,3 octanedione has been associated with the flavor described
307 as "mushroom flavor" (Schindler, Krings, Berger, & Orlien, 2010). This flavor was among the sensory
308 defects perceived by the panelists in milk samples of the present study. The origin of 2,3
309 octanedione has not been largely investigated and it is generally accepted that it can derive from
310 the n-6 fatty acid oxidative process. However, another interesting pathway for 2,3 octanedione
311 formation has been described by Pompizzi et al. (2000). These authors suggested that this

312 compound can derive from the photo-oxidative degradation of furan fatty acids present in some
313 foods. The presence of these acids has been demonstrated in different oils and fats and, in
314 particular, the dimethyl pentyl furan fatty acids seem to be the major constituents of this class of
315 bioactive furan fatty acids in milk, originated from the cow's feed (Wendlinger & Vetter, 2014). The
316 very low relative abundance of 2,3 octanedione in milk stored in the dark and its increase under
317 light exposure withstands the theory that this volatile is produced by the cycloaddition of singlet
318 oxygen (generated after light exposure) to a dimethyl pentyl furan with the formation of a bicyclic
319 furan endoperoxide that leads to the formation of the di-ketone through a double pathway.
320 Remarkably, no sulfur-containing compounds were detected in the current study even under the
321 most stressing storage conditions tested. This finding is in accordance with previous works reporting
322 that volatile sulfur compounds such as hydrogen sulfide, dimethyl disulfide, methanethiol, are
323 typical flavor components of milk heated at conditions more severe than those used here
324 (Beauchamp et al. 2014; Jo, Carter, Barbano, & Drake, 2019). These compounds originate from
325 degradation of sulfur-containing amino acids and, being the levels of free methionine negligible, an
326 extensive denaturation of whey proteins would be required for their formation. Likely, the lack of
327 interfering sulfur-containing compounds could have made it easier for the panelists to perceive
328 other light- or storage-induced compounds and odors, as it is further discussed.

329 **3.6 Sensory analysis**

330 Results obtained from sensory analysis are displayed in the whisker plots in Figure 3 and in Table
331 S4. No major changes were perceived by the panelists in the control samples (dark storage) during
332 the first 10 days of storage and, in samples stored up to 13 days, the average score for the "typical
333 milk flavor" slightly decreased, from 4.3 to 3.7. These results confirmed that appropriate storage
334 conditions (dark, uninterrupted refrigeration) allow to retain the natural sensory characteristic of
335 fresh milk along the shelf life indicated by the manufacturer or even longer. Remarkably, the

336 average score was below 2 for all the selected descriptors of off-flavors. As expected, light exposure
337 of milk influenced the perception of these last, although to a different extent and sometimes
338 depending on the age of milk. The “mushroom” flavor reached the highest scores among the
339 descriptors. Flavor intensity increased with the increase of lighting time, but the variability of
340 perception was very high among panelists. For the “garlic, cabbage” flavor, significant increases
341 were observed after 6 or 12 hours of lighting, even if the average score did not exceed 2.6. The
342 “rancid, soapy” flavor was less influenced from light exposure and showed only a slight variation
343 with the increase of lighting, more evident after 7 days of storage in the dark.

344 Overall, panelists’ evaluation was more strongly influenced by exposure to light than by storage
345 time, as previously observed by other authors (Martin et al., 2016). The PLS analysis indicated the
346 “mushroom” descriptor was mainly affected by the light exposure, while the “typical milk flavor” by
347 milk storage. Moreover, these two descriptors were the most important ones (VIP=1.30 and
348 VIP=1.17, respectively for mushroom and typical milk flavor), followed by “garlic, cabbage”
349 (VIP=0.75) and “rancid, soap” (VIP=0.61). Neither sulphur nor eggy off-flavors were detected.
350 Indeed, as already mentioned, these unpleasant odors mainly occur in high-temperature heated
351 milk, where whey proteins are extensively denatured (Jo et al., 2019).

352 **3.7 Issues and Perspectives for defining the shelf life of lighted pasteurized milk**

353 In order to analyze the overall sensitivity of milk to the light exposure also considering its storage, a
354 Principal Component Analysis (PCA) was performed including all the chemical and sensory data
355 obtained at the different sampling times. Figure 4a shows the score chart of the first two principal
356 components and the Hotelling T2 Ellipse line with 95% confidence level. These components
357 accounted for the 84% of the total variance. Samples are labelled with the shelf life time (t_x) both in
358 the dark (marked as 0) and after light exposure (marked as follows: 1=6h, 2=12h, 3=18h). PC1 (that
359 accounts the 60% of the total variance) described the effect of light exposure, making a distinction

among samples stored under light for 6 hours (on the right of the graph, positive PC1s) and for 12 and 18 hours (on the left of the graph, negative PC1s). PC2 (that accounts the 24% of the total variance) described the effect of the milk storage, distinguishing samples retained up to 7-8 days from those exceeding that time.

The loadings represented in Figure 4b reveal that samples are described by different quality attributes considering the time of light exposure on the shelf. Variables that decreased as function of the light exposure presented positive values, while those that increased showed negative values. A high and positive correlation (r=0.977) has been found between the sensory descriptor "mushroom" and the compound 2,3 octanedione supporting the contribution of this compound to the light-dependent defect.

The loadings represented in Figure 4b reveals that samples are described by different quality attributes considering the time of light exposure on the shelf. Variables that decreased as function of the light exposure presented positive values, while those that increased their values when exposed to light showed negative values. Among variables, a high and positive correlation (r=0.977) has been found between the sensory descriptor "mushroom" and the compound 2,3 octanedione supporting the contribution of this compound to the light-dependent defect. A positive correlation has been also found between the descriptor "rancid" and the hexanal (r=0.763). The involvement of RF and FMN in the appearance of sunlight flavor is also supported by the high and inverse correlation values between these two compounds and the sensory descriptors "mushroom" (-0.832 and -0.888 for RF and FMN, respectively), "rancid" (-0.736 and -0.771 for RF and FMN, respectively) and "garlic, cabbage" (-0.760 and -0.881 for RF and FMN, respectively). In fact, the inverse correlation means that the sensory defects perceived by the trained panel increased as RF and FMN content decreased.

383 The exposure to the fluorescent light for 6 hours did not contribute to modify significantly the
384 quality of pasteurized milk despite the storage of the milk. Samples exposed for 18 hours showed
385 the most negative PC1 values.
386 The PC1 scores obtained from the PCA were further plotted against the lighting time of exposure.
387 Therefore, the multivariate kinetic parameters *k* were obtained~~,~~ in order to evaluate the reaction
388 order and the degradation rate, taking the contribution of each variable into account (Pedro &
389 Ferreira, 2006). In Table 2 the multivariate rate constants obtained for the multivariate quality index
390 Q at each sampling time are shown. All the decay reactions under light followed a zero order kinetic
391 and the negative values of the rate constant k_m evidenced that the overall quality Q of milk under
392 light did not decrease as function of the storage time of milk. However, only after 10 days of storage
393 the quality decay rate under light changed in a significant way, demonstrating that the sensitivity of
394 pasteurized milk to light exposure is not affected by storage.
395 The information collected in this study has been also used to tentatively identify define the shelf life
396 or, better, "*the length of time a product may be stored without becoming unsuitable for use or*
397 *consumption*" (Guillet & Rodrigue, 2009). In the case of pasteurized milk in clear PET bottles, this
398 time may represent the maximum time beyond which the photo-oxidative damage increases, with
399 loss of vitamins and the risk of leading to perception of off-flavours. In fact, as it is hardly feasible to
400 avoid light exposure of milk during the sale step at retailer level, a possible strategy would be to
401 optimize the light exposure as function of the residual shelf life of the product.
402 The multivariate approach used in this study allowed to identify the positioning of a reference
403 sample in the matrix (REF, in Figure 4a) characterized by an acceptable value for each of the quality
404 variable here considered. Acceptable reference values were established as follows: the total
405 bacterial count of 10^6 CFU/mL was taken as a prudential and not mandatory limit, while the average
406 values obtained for the samples stored in the dark were taken as for flavins, volatile compounds

and proteose-peptones. Finally, the score equal to 4 was considered for the sensory attribute "typical milk flavor", and equal to 2 for "mushroom", "garlic and cabbage" and "rancid and soap". Thus the loading matrix was used to calculate the multivariate cut-off criteria, that is the maximum acceptable score for each time-related PC. With this approach, the resulting cut-off points were equal to -1.4 and 1.9 for PC1 and PC2 scores, respectively. Being PC1 related to the light exposure, the cut-off point of -1.4 was considered and from this limit a maximum of 12 hours under the fluorescent light was derived (vertical dotted line in Figure 4a) for pasteurized milk during its shelf life. Based on our data, RF content did not significantly change in milk under these conditions. Since milk is one of the main source of RF, and this vitamin plays important biological functions (i.e. regulation of cell growth and biological redox reactions) the proposed acceptability criteria allow to preserve also the nutritional value of milk due to the important functions RF plays (Giménez, Gagliardi, & Ares, 2017).

The second component (PC2) accounted for 24% of the variation in the original data set and was storage-time related (Figure 4a). As suggested by Gimenez et al. (2017), the time-related component can be used to define the end of shelf life on the basis of the multivariate failure criterion. Therefore, considering the PC2 score for the cut-off point as the limit after which the quality of milk cannot be considered acceptable and given a total of 12 hours under fluorescent light, the shelf life of pasteurized milk in PET bottles could be set at 10 days (horizontal dotted line in Figure 4a), instead of 7 days as indicated by the manufacturer.

Finally, the failure exposure time was correlated to the emission spectrum of the fluorescent lamp, in order to estimate the maximum energy supported by the pasteurized full-fat milk during its exposure on the shelf. The profile of cumulative irradiance (W m^{-2}) emitted by the fluorescent lamp was recorded by the spectrophotometer placed in the same positions of the bottle and estimated within the three main spectral regions (blue-violet, 360-490 nm; green-yellow, 491-590 nm; orange-

431 red, 591-780 nm) (Table S5). Wold et al. (2015) demonstrated that not only wavelengths lower than
432 500 nm can degrade RF, decreasing the quality and the nutritional value of milk, but also longer
433 visible wavelengths can induce the formation of sunlight flavour in milk due to the presence of
434 chlorophylls and tetrapyrroles that absorb in the red region. Thus, based on the milk
435 photosensitivity, the evaluation of lamp emission characteristics in terms of irradiance in different
436 regions of the visible spectrum could orientate in optimizing light exposure conditions of milk in the
437 retail case. On the basis of the spectral irradiance of the lamp in the different positions of the shelves
438 and the maximum time under light estimated for the full-fat pasteurized milk (12h), the radiant
439 exposure of the lamp for 1 cm^2 of surface was calculated for the three intervals of the visible
440 spectrum (Table S6). Lamps characterized by radiant exposure higher than the maximum for each
441 visible spectrum interval could accelerate milk storage during 12 hours of storage on the lighted
442 shelf, forcing the exposition to be reduced.
443 The adoption of monochromatic lights or, alternatively, bottles of coloured plastic materials could
444 be expensive and not always achievable both for economic and recycling reasons. However, the
445 identification of maximum levels of energy emitted for surface unit could be exploited to develop
446 tuneable white light lamps and/or optimize the permanence on the shelf of pasteurized milk in clear
447 PET bottles. Multi-layer carton with the aluminium foil or plastics containing light-protective
448 additives fully prevent milk photo-oxidation (Stancik et al., 2017; Wang et al., 2018). However, the
449 urgent necessity to simplify the packaging structures to support recyclability, especially for fossil-
450 based polymers like PET, it often clashes with the need to guarantee the quality of food for longer
451 time to avoid unnecessary waste.

452 **4. Conclusion**

453 The chemical and sensory markers monitored during the storage of milk showed a good stability of
454 the product. The sensitivity of milk to light was further evidenced as well as the light-dependent

455 negative effects causing losses of nutritional value, as riboflavin was degraded, and changes in the
456 sensory profile. Remarkably, the estimated shelf life turned out to be longer than expected by the
457 manufacturer for the milk considered in this study. This occurs when both storage temperature and
458 light exposure, in terms of both duration and lamp emission, are properly managed. As a
459 consequence, the overall quality of milk can be maintained. The application of new storage-on-shelf
460 studies based on the multivariate relationship among the risky variables for the target product
461 allowed the definition of suitable cut-off criteria and, considering the light source characteristics, it
462 can be useful in estimating the shelf life under specific and real conditions. The proper choice of
463 lamp (i.e. spectral irradiance) combined with an adequate logistic in the retail (i.e. rotation of milk
464 bottles on the shelves, knowledge of radiant exposure of a food) can support sustainable packaging
465 solutions preserving the quality of a sensitive food.

466

467 **Declarations of interest**: none.

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598

Figures captions

Figure 1. a) Emission spectra of the fluorescent lamp itself (grey line) and after the insertion of the PET film (black line) **b)** Emission spectra of the fluorescent lamp in three different positions of the shelf: center (black line), a halfway point (dotted line) and shelf end (grey line).

Figure 2. Pentanal (a), hexanal (b), heptanal (c), octanal (d), 2-butanone (e) and 2,3-octanedione (f) in milk stored at dark (0h; black bars)) and after 6 hours (grey bars) and 18 hours (light gray bars) under light exposure, at different storage times (days). Results are expressed as ratio between the peak area of each compound and the peak area of the internal standard (A/Ais); the error bars indicate the standard deviation of triplicate analysis. Different lowercase letters indicate a significant difference for increasing time of exposure to light within each volatile ($p < 0.05$). Different capital letters indicate a significant difference increasing time of storage ($p < 0.05$).

Figure 3: Whisker plots for (a) typical milk flavor (b) mushrooms, (c) garlic and cabbage and (d) rancid and soap descriptors selected for the sensory analysis. Data are presented with standard deviation.

Figure 4. Score plot (a) and Loading plot (b) of data collected during the milk storage and light exposure. Samples are labelled with the shelf life time (t_x) both in the dark (marked as _0) and after light exposure (marked as follows: _1=6h, _2=12h, _3=18h).

Table 1. Content of studied flavins in milk stored in the dark (0 hours of light exposure) and after 6, 12 and 18 hours of light exposure (n=4), at different storage times (n=5). *: data referred to milk at time 0.

	Light exposure	Storage time (days)				
flavin	(hours)	2	3	7	8	13
riboflavin (mg/L) [3.34±0.11]*	0	3.31±0.12 aA	3.33±0.14 aA	3.30±0.11 aA	3.22±0.12 aA	3.20±0.17 aA
	6	3.19±0.13 aA	3.18±0.04 abA	3.19±0.02 aA	3.19±0.23 aA	3.16±0.16 aA
	12	3.08±0.07 aA	2.98±0.14 bA	2.98±0.18 bA	3.14±0.05 aA	2.93±0.06 bA
	18	2.88±0.08 bA	2.73±0.31 cA	2.99±0.05 bA	2.89±0.09 bA	2.79±0.16 bA
flavin mononucleotide (μg/L) [125.4±1.7]*	0	123.8±7.4 aA	122.4±2.4 aA	121.9±4.3 aA	121.2±2.9 aA	118.5±3.3 aA
	6	115.7±9.1 aA	112.0±6.8 abA	115.9±2.0 aA	118.7±5.5 aA	115.2±3.0 aA
	12	106.0±4.7 aA	102.3±1.7 abA	103.0±0.9 bA	113.0±2.0 aB	105.0±1.8 bA
	18	97.4±4.9 bA	89.7±6.6 bA	101.3±5.0 bA	97.8±5.0 bA	97.4±5.9 bA
lumiflavin (μg/L) [0.3±0.1]*	0	0.4±0.1 aA	0.4±0.1 aA	0.4±0.2 aA	0.4±0.1 aA	0.4±0.2 aA
	6	0.7±0.2 abA	0.8±0.0 bA	0.8±0.3 aA	0.7±0.2 bA	0.8±0.2 bA
	12	1.3±0.2 bA	1.7±0.4 cA	1.2±0.1 bA	1.1±0.3 bcA	0.9±0.3 bA
	18	1.4 ±0.1 bA	2.0±0.3 cA	1.6±0.0 cA	1.5±0.2 cA	1.3±0.2 bcA
lumichrome (μg/L) [0.7±0.4]*	0	0.7±0.4 aA	1.2±0.3 aA	2.2±0.6 aA	3.2±0.5 aB	3.6±1.3 aB
	6	2.1±0.7 bA	4.2±2.1 bA	8.9±3.2 bA	5.9±2.1 aA	5.6±2.1 aA
	12	7.1±2.3 cA	8.2±4.1 cA	7.7±1.2 bA	7.8±1.5 bA	7.9±1.9 aA
	18	14.3±1.0 dA	13.2±4.9 dA	15.8±3.4 cA	15.0±1.7 cA	12.9±1.3 bA

Data are expressed as mean ± standard deviation.
Different lowercase letters indicate a significant difference for increasing time of exposure to light within each flavin (n=4) ($p < 0.05$).
Different capital letters indicate a significant difference at increasing storage time (n=5) ($p < 0.05$).

Table 2. Kinetic multivariate models for light exposure ($Qt=-k_m*t+Q_0$) during storage, multivariate rate constants k_m and correlation coefficients r between measured and predicted values.

Storage time (d)*	$Qt=-k_m*t+Q_0$ (t, hour)	k_m (PC1 score h^{-1})	r
2	Qt=-0.44*t+4.5	-0.44	0.99
3	Qt=-0.43*t+3.3	-0.43	0.99
7	Qt=-0.44*t+3.9	-0.44	0.99
8	Qt=-0.44*t+4.0	-0.46	0.97
10	Qt=-0.48*t+4.0	-0.40	0.98
13	Qt=-0.31*t+1.1	-0.31	0.97
average	Qt=-0.42*t+3.3	-0.42	