

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

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14 **Abstract**

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

26

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

## 29 1. Introduction

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

47 Producing commercial pasteurized milk with a longer shelf life has measurable economic  
48 advantages for manufacturers and allows reducing food waste due to premature spoilage.  
49 Extending the shelf life, however, increases the time the bottled milk may stay on the display  
50 counter in the shops. Although the low temperature slows down both bacterial growth and  
51 enzymatic activities, when milk is exposed to light at wavelengths in the UV-visible range, such as  
52 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<sub>2</sub>) 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 ( $1.2 \times 10^4$  CFU/mL)  
104        and somatic cell count ( $2.17 \times 10^5$  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 ( $5 \pm 1^\circ\text{C}$ ) 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 ( $4 \pm 1^\circ\text{C}$ ) 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 ( $4 \pm 1^\circ\text{C}$ ) 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 ( $5 \pm 1^\circ\text{C}$ ) and vertical retail case with four shelves  
120        was used (Arrigoni, Italy) and the display was placed in a dark and conditioned room at  $25^\circ\text{C}$ ,  
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 ( $\text{W m}^{-2}$ ) 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 5000g for 20 minutes. The supernatant was filtered through a 0.22  $\mu\text{m}$  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

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

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

## 157 **2.6. Determination of riboflavin and flavones**

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

## 167 **2.7. Determination of volatile compounds**

168 The volatile compounds were sampled by a headspace solid phase micro extraction (SPME)  
169 technique followed by gas chromatography–mass spectrometry (GC-MS). Ten mL of milk sample  
170 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  $\mu\text{m}$  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  $\mu\text{m}$ ; 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 ( $A/A_{is}$ ), 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

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

## 198 **2.9. Statistical analysis**

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

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

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

218 relationship with light exposure and storage time, while the loadings to reveal the key attributes  
219 responsible for the product degradation, under the different conditions of storage. At each sampling  
220 time, the relationship between the multivariate quality parameter  $Q$  (represented by each PC1 value  
221 obtained in the PCA) and the time of exposure  $t$  was derived as described by the following equation  
222 (1):

$$223 \quad \frac{dQ}{dt} = k_m Q^n$$

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

225

## 226 **3. Results and Discussion**

### 227 **3.1 Characterization of the lighting system**

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

### 234 **3.2 Oxygen consumption**

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

240 into the bottle, maintaining quite constant the gas concentration in the milk. Light exposure had an  
241 important influence on oxygen consumption in milk samples, as recently described (Wang et al.,  
242 2018). Oxygen concentration decreased exponentially with increasing time of light exposure, being  
243 values halved after 18 hours. The oxygen consumption followed a first order kinetic independently  
244 of the milk age, and the rate of consumption was significantly higher at 13d of storage ( $p=0.0387$ )  
245 in comparison to 3d (Figure S2). Interestingly, as storage increased, the curve of oxygen  
246 consumption rate moved towards lower oxygen concentrations, although the content of dissolved  
247 oxygen after 6 and 12 hours of light exposure was not significantly different among the samples  
248 with 3 and 8 days of storage ( $p>0.05$ ).

### 249 **3.3 Proteolysis progress**

250 Proteose peptones (PP) directly derive from the proteolytic activity of plasmin on  $\beta$ -casein. Since  
251 plasmin is stable to milk pasteurization, the progress of this proteolytic activity was associated to  
252 the quality decay of pasteurized milk during storage (Murphy et al., 2016). For this reason, a PP  
253 concentration not higher than 900 mg/L was proposed for characterizing pasteurized milk that  
254 maintains acceptable quality during the shelf life (De Noni, Pellegrino, Cattaneo, & Resmini, 2007).  
255 In long stored pasteurized milk, PP are further degraded into small peptides (SP) by bacterial  
256 proteases (Cattaneo et al., 2014). Based on this knowledge, both PP and SP were taken in this study  
257 as descriptors of proteolysis progress, thus of milk storage itself, owing to the fact that their content  
258 was not influenced by light exposure regardless the age of milk ( $p > 0.79$  for PP and  $p > 0.99$  for SP)  
259 (Figure S3). As expected, the content of PP increased linearly ( $r=0.99$ ) during storage (data not  
260 shown), as a consequence of plasmin activity, and approached the threshold value of 900 mg/L at  
261 13 days of dark storage, that is 6 days beyond the shelf life indicated by the manufacturer. In  
262 contrast, the content of SP did not increase significantly due to the good microbiological quality of  
263 the raw milk used by the manufacturer.

264 The lack of a relevant microbial protease activity also explains why the content of free amino acids  
265 did not change significantly during milk storage. In fact, the total levels of free amino acids were  
266 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.  
267 These data were not significantly different ( $p > 0.05$ ) from the initial (day 1) level of 82.1±1.0 mg/L.  
268 In particular, no changes were observed in free methionine content that was always lower than 0.1  
269 mg/L (not shown), in agreement with literature data (Pellegrino et al., 2015). These low levels  
270 suggest that, although this amino acid is sensitive to light oxidation (Min & Boff, 2002), its  
271 contribution to the development of sulfur-containing compounds responsible of off-flavors can be  
272 considered negligible. No free cysteine was detected.

### 273 **3.4 Riboflavin and other flavones**

274 The content of both riboflavin (RF) and flavin mononucleotide (FMN) remained unchanged in the  
275 control samples (dark storage) up to 13 days (Table 1), confirming the stability of these compounds  
276 in absence of a light source (Sheraz, Kazi, Ahmed, Anwar, & Ahmad, 2014). Contrarily, both flavins  
277 slowly degraded as the cumulative time of light exposure increased, reaching levels significantly  
278 different from the control after 18 hours of lighting. No flavin adenine dinucleotide was detected,  
279 while a higher concentration of RF than those found in Fracassetti et al. (2018) was determined.  
280 Both RF and FMN are involved in photo-oxidation reactions (Choe, Huang, & Min, 2005). The light  
281 exposure can cause the cleavage of the ribityl group of excited triplet RF leading to the production  
282 of several compounds, i.e. lumichrome (LC) and, to a lesser extent, lumiflavin (LF) (Sheraz et al.,  
283 2014). These two compounds are also photosensitizers and seem to have aptitude to form singlet  
284 oxygen comparable to those of RF, thus may equally contribute to decay of sensory quality of milk  
285 (Huang, Kim, & Min, 2006). Both LF and LC contents increased upon light exposure, with a significant  
286 difference from the control observed even after 6 hours lighting. Little increase of LF was found in  
287 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 T<sup>2</sup> 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

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

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

370 The loadings represented in Figure 4b reveals that samples are described by different quality  
371 attributes considering the time of light exposure on the shelf. Variables that decreased as function  
372 of the light exposure presented positive values, while those that increased their values when  
373 exposed to light showed negative values. Among variables, a high and positive correlation ( $r=0.977$ )  
374 has been found between the sensory descriptor “mushroom” and the compound 2,3 octanedione  
375 supporting the contribution of this compound to the light-dependent defect. A positive correlation  
376 has been also found between the descriptor “rancid” and the hexanal ( $r=0.763$ ). The involvement  
377 of RF and FMN in the appearance of sunlight flavor is also supported by the high and inverse  
378 correlation values between these two compounds and the sensory descriptors “mushroom” (-0.832  
379 and -0.888 for RF and FMN, respectively), “rancid” (-0.736 and -0.771 for RF and FMN, respectively)  
380 and “garlic, cabbage” (-0.760 and -0.881 for RF and FMN, respectively). In fact, the inverse  
381 correlation means that the sensory defects perceived by the trained panel increased as RF and FMN  
382 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

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

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

426 Finally, the failure exposure time was correlated to the emission spectrum of the fluorescent lamp,  
427 in order to estimate the maximum energy supported by the pasteurized full-fat milk during its  
428 exposure on the shelf. The profile of cumulative irradiance ( $W\ m^{-2}$ ) emitted by the fluorescent lamp  
429 was recorded by the spectrophotometer placed in the same positions of the bottle and estimated  
430 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<sup>2</sup> 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 ( $A/A_{is}$ ); 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.

flavin	Light exposure		Storage time (days)				
	(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 ( $Q_t = -k_m * t + Q_0$ ) during storage, multivariate rate constants  $k_m$  and correlation coefficients  $r$  between measured and predicted values.

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